MEDICAL LIBRARY

JUN 7 1948

CANCER RESEARCH

VOLUME 7
NUMBER 8
AUGUST, 1947

A MONTHLY JOURNAL
OF ARTICLES AND ABSTRACTS
REPORTING CANCER RESEARCH

CONTENTS

MARGARET ARMSTRONG and ARTHUR HAM. Effects, Particularly Anemia, Produced in Chicks by Growth in Their Yolk Sacs of Mouse Mammary Tumors 481

HARRY S. N. GREENE. The Use of the Mouse Eye in Transplantation Experiments 491

HARRY S. N. GREENE, B. L. NEWTON, and ALBERT A. FISK. Carcinoma of the Vaginal Wall in the Rabbit 502

W. F. DUNNING, M. R. CURTIS, and A. SEGALOFF. Strain Differences in Response to Diethylstilbestrol and the Induction of Mammary Gland and Bladder Cancer in the Rat 511

CYRUS P. BARNUM, ZELDA B. BALL, and JOHN J. BITTNER. Partial Separation of the Mammary Tumor Milk Agent and a Comparison of Various Sources of the Agent 522

WERNER G. JAFFÉ. The Response of Mice to the Simultaneous Application of Two Different Carcinogenic Agents 529

F. E. KELSEY and ALEXANDER BRUNSCHWIG. Studies on Drug Absorption. Fixation of Quinine by Neoplastic and Non-Neoplastic Tissues 531

E. W. MCHENRY, E. M. SEMMONS, R. PEARSE, and E. G. MEYER. Observations on the Ketosteroid Content of Urine from Patients with Prostatic Carcinoma and Adenoma 534

MAHMOUD AHMED AFIFI. Cancer Mortality in Egypt 537

BOOK REVIEW 547

THE OFFICIAL ORGAN OF THE
AMERICAN ASSOCIATION FOR CANCER RESEARCH, INC.

CANCER RESEARCH

This journal is sponsored by the American Association for Cancer Research, Inc.; The Anna Fuller Fund; Cancer Research Division, Donner Foundation, Incorporated; The Jane Coffin Childs Memorial Fund for Medical Research; and the Elsa U. Pardee Foundation.

Editors

BALDUIN LUCKÉ, *Editor* — MORTON MCCUTCHEON, *Associate Editor*
E. W. SHRIGLEY, *Editor, Abstracts Section*

Advisory Board

MILDRED W. S. SCHRAM, *Chairman*
W. W. ALLEN — JAMES B. MURPHY
S. BAYNE-JONES — M. J. SHEAR
GEORGE M. SMITH

Editorial Committee

JAMES B. MURPHY, *Rockefeller Institute, Chairman*

J. J. BITTNER, *University of Minnesota Medical School*
A. BRUNSCHWIG, *Memorial Hospital, New York City*
E. V. COWDRY, *Barnard Free Skin and Cancer Hospital*
D. R. COMAN, *University of Pennsylvania School of Medicine*
P. R. CUSTER, *Presbyterian Hospital, Philadelphia*
L. I. DUBLIN, *Metropolitan Life Insurance Company*
F. DURAN-REYNALS, *Yale University School of Medicine*
G. FAILLA, *College of Physicians and Surgeons, New York City*
L. F. FIESER, *Harvard University*
A. J. FRENCH, *University of Michigan, Ann Arbor*
J. FURTH, *Veterans' Administration Hospital, Dallas, Texas*
W. U. GARDNER, *Yale University School of Medicine*
H. S. N. GREENE, *Yale University School of Medicine*
J. P. GREENSTEIN, *National Cancer Institute*
F. L. HAVEN, *Strong Memorial Hospital*
J. L. HARTWELL, *National Cancer Institute*
J. G. KIDD, *Cornell University Medical College*
E. C. MACDOWELL, *Carnegie Institution of Washington*
G. B. MIDER, *Strong Memorial Hospital*
E. G. MILLER, JR., *College of Physicians and Surgeons, New York City*
J. J. MORTON, JR., *Strong Memorial Hospital*
E. H. QUIMBY, *College of Physicians and Surgeons, New York City*
H. L. STEWART, *National Cancer Institute*
G. H. TWOMBLY, *Memorial Hospital, New York City*
S. WARREN, *Harvard Medical School*

Abstractors

C. AUGER
E. B. BARBER
W. A. BARNES
M. BELKIN
R. BRIGGS
W. J. BURDETTE
B. R. BURMESTER
A. CORNELL
H. J. CREECH
Z. DISCHE
L. J. DUNHAM
A. HADDOW
J. B. HAMILTON
F. L. HAVEN
J. HEIMAN
I. HIEGER
G. H. HOGEBOOM
M. E. HOWARD
R. A. HUSEBY
R. JAHIEL
R. N. JONES
H. S. KAPLAN
E. L. KENNAWAY
J. G. KIDD
A. KIRSCHBAUM
M. LEDERMAN
M. L. LEVIN
V. J. LONGO
R. J. LUDFORD
V. F. MARSHALL
W. V. MAYNEORD
J. L. MELNICK
A. MELTZER
C. R. NOBACK
M. H. PESKIN
C. A. PFEIFFER
K. R. PORTER
L. W. PRICE
E. H. QUIMBY
E. C. RICHARDSON
D. SHEMIN
R. E. SNYDER
E. E. SPROUL
M. TAFFEL
F. L. WARREN
G. W. WOOLEY

Published by Cancer Research Division, Donner Foundation, Incorporated
Publication Office, 317 Maynard Street, Ann Arbor, Michigan

The annual subscription rates for one volume are: To members of the American Association for Cancer Research, Inc., $5.00; to others and to libraries, institutions, and organizations, domestic, $7.00; Canadian, $7.50; Foreign, $8.00. Single copies, $1.00. Business communications, remittances, and subscriptions should be addressed to Dr. Theodore P. Eberhard, Business Manager, Jefferson Hospital, 10th and Sansom Streets, Philadelphia 7, Pa.

No responsibility is accepted by the Committee, by the Board, or by the Publishers of *Cancer Research* for opinions expressed by contributors.

Entered as second class matter December 16, 1946, at the Post Office at New Haven, Conn., under the Act of March 3, 1879. Application pending transfer to Ann Arbor, Mich.

Copyright, 1947, by Cancer Research Division, Donner Foundation, Incorporated.

CANCER RESEARCH

A MONTHLY JOURNAL OF ARTICLES AND ABSTRACTS REPORTING CANCER RESEARCH

VOLUME 7 AUGUST, 1947 NUMBER 8

Effects, Particularly Anemia, Produced in Chicks by Growth in Their Yolk Sacs of Mouse Mammary Tumors

Margaret Armstrong, M. D., and Arthur Ham, M. B.

(*From the Department of Anatomy, University of Toronto, Toronto, Canada*)

(Received for publication April 2, 1947)

INTRODUCTION

Heilman and Bittner (7) have reported that the serial transfer of mouse mammary tumors in the yolk sacs of fertile eggs is associated with their manifesting an increased lethal effect on the developing chicks. The first purpose of this study was to perform sufficient experiments of a similar nature to provide confirmation or denial of this finding. The second purpose was, if confirmation were obtained, to learn, from the study of the tissues and fluids of the chicks concerned, the cause or causes of their deaths. It was thought that precise information on this point might allow some proper inference to be drawn about the nature of the increased lethal effect, and that this in turn might be of some relevance to the general problem of malignancy.

MATERIAL AND METHODS

Fertile eggs from a pure strain of White Leghorns were incubated at 99.5° F. (wet bulb—83° F.). Yolk sacs were inoculated with mouse tumor tissue on the fourth day of incubation, essentially by the method of Taylor, Hungate and Taylor (14). Tumors resulting from this procedure were serially transferred in eggs, *i.e.*, when the first tumors were obtained in eggs they were recovered and injected into other eggs, then when tumors grew in these they were injected into still other eggs, and so on. The details regarding the tumors so treated are given, with the observations made on them.

For primary inoculations of eggs, tumor tissue obtained directly from a mouse was minced, and then diluted to 5 times its volume with physiological saline. For subsequent egg inoculations, yolk sac tumor tissue was diluted to the same extent, not with saline, but with a mixture of yolk, amniotic fluid, allantoic fluid and blood from the egg in which the tumor was grown. This dilution was consistently adhered to except for a separate experiment which was performed to test the effects of different dilutions. The details of the dilutions employed in this separate experiment are presented later.

To obtain undiluted blood for hemoglobin and blood sugar estimations the shell over the air space was removed. The shell membrane so exposed was wetted to make it transparent. The large allantoic vein beneath it, then being visible, was grasped with forceps pushed through the membrane, and drawn out. The blood was taken from it with a 0.25 cc. syringe provided with a No. 25 gauge needle. Hemoglobin estimations were made with the Evelyn colorimeter by Evelyn's method. Because the chick's erythrocytes are nucleated, and hence difficult to count apart from leukocytes and thrombocytes, and because leukocytes and thrombocytes comprise a very insignificant proportion of the total blood cells, particularly at this stage of development, total blood counts were made and employed for color index determinations. Blood films were prepared and stained with Hasting's stain. Some blood sugar determinations were also made by the method of Miller and Van Slyke (11).

Sections of formalin-fixed tissues and organs of chicks were cut in paraffin and stained with hematoxylin and eosin.

OBSERVATIONS

1. On the Enhanced Lethal Effect of Tumors after Serial Egg Passage

Four tumors that we named and treated as follows were studied: ET, a spontaneous mam-

This work was carried out by means of grants from the Ontario Cancer Treatment and Research Foundation, to which the authors are most grateful. The authors wish also to thank Dr. Alice Gray for specialized help on several occasions and George Ross for general technical assistance. Acknowledgement is made to the Department of Physiology, University of Toronto, for making some blood sugar determinations.

mary carcinoma from a C3H female mouse, was transplanted once into another C3H mouse and then into eggs in which it has been transferred 40 times to date; ECT, a spontaneous mammary tumor from a female C3H mouse, was injected directly into eggs, where it has undergone 8 transfers; EAT 1, a spontaneous mammary tumor from a mouse of the A strain, was injected directly into eggs, where it underwent 12 transfers before it was lost; EAT 2, a spontaneous mammary tumor from a female mouse of the A strain, was transplanted once into another A strain mouse and then into eggs, where it has undergone 41 transfers.

The *early* (first 10 days of incubation) mortality rate of chicks varied neither with the number of times the tumor had been transferred in eggs nor with the particular tumor grown; it remained remarkably constant (between 21 and 25 per cent) except for a period during which the war-time motor in our incubator became inefficient with regard to circulating the air and so caused a general increase in the mortality rate of both inoculated and normal eggs, a state of affairs that returned to normal after the installation of a new motor.

Cultures in thioglycollate medium (Difco Bacto-Fluid) showed that only about 3 per cent of the early deaths could be attributed to bacterial infection. Operative interference, it is presumed, was responsible for the remainder. Our results in this respect seem to be similar to those of King, Ball and Menefee (9) who observed some early deaths not due to contamination.

The *late* (from the 11th to 17th day of incubation) mortality rate was, in the early egg transfers of all 4 tumors, consistently low. But, with the continued serial transfer of tumors in eggs, the late mortality rate became progressively greater and the time of death progressively earlier.

Our observations regarding these two points on ET, the tumor that we have studied most, are recorded graphically in Fig. 1. It is to be noted that the results from several consecutive transfers, rather than from each transfer, have been plotted; this is to facilitate the reading of the graph and also to assure that each curve was plotted from a large number of eggs. It is to be noted also that no results are plotted for the 11th to 17th transfers; this omission is due to the incubator trouble described above, which, being difficult to diagnose, was not remedied until the 16th transfer, and resulted in too few eggs being carried through this period to provide figures of statistical significance. However, in the instance of normal eggs, it was observed that the incubator defect affected the early rather than the late mortality rate, hence, as eggs inoculated after the 11th transfer began to die in increasing numbers in the later stages of incubation, and as they revealed pathological stigmas found afterward to be characteristic of the increased lethal effect of the tumor, it seems justifiable to assume that the first signs of the enhanced lethal effect of ET occurred after its 11th transfer and, as is indicated in Fig. 1, was well established by the 18th transfer.

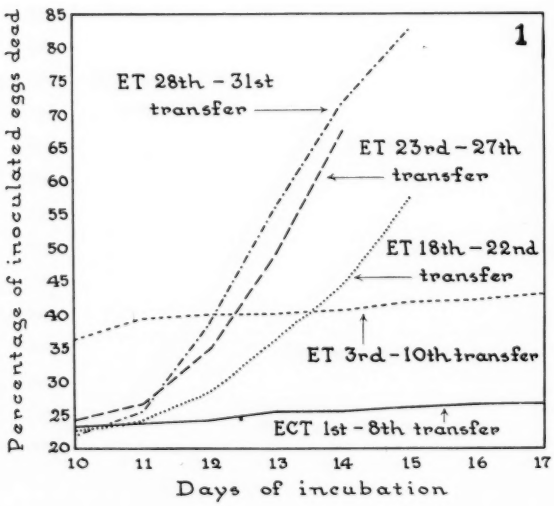

1
Percentage of inoculated eggs dead
Days of incubation
ET 28th - 31st transfer
ET 23rd - 27th transfer
ET 18th - 22nd transfer
ET 3rd - 10th transfer
ECT 1st - 8th transfer

FIG. 1.—Effect of number of times tumors have been transferred in eggs on their lethal effect. ECT 1-8 was plotted from 164 eggs, ET 3-10 from 219 eggs, ET 18-22 from 599 eggs, ET 23-27 from 667 eggs, and ET 28-31 from 564 eggs.

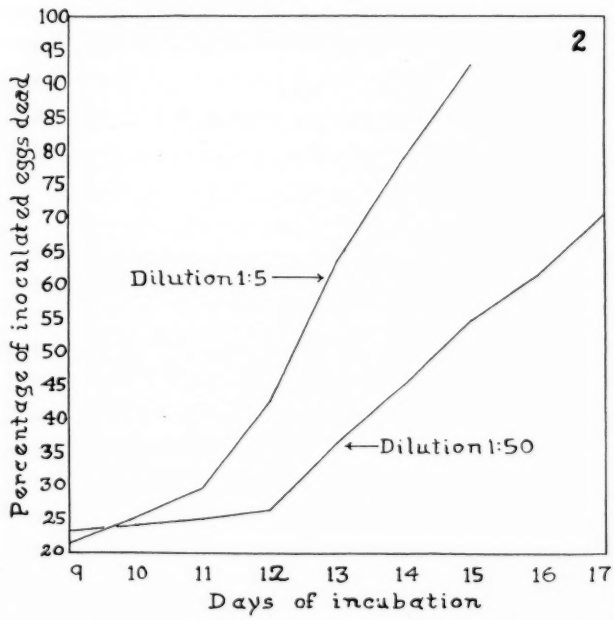


FIG. 2.—Effect of amount of tumor tissue (derived from tumors of many serial passages) with which eggs are inoculated on lethality of tumor subsequently developing.

In the serial transfer of ET in eggs, 99 per cent of the inoculated eggs developed yolk sac tumors.

The late mortality rate of eggs inoculated with ECT, another C3H tumor, is also shown in Fig. 1. From our experience its curve may be taken as typical of the late mortality rate of eggs inoculated with mouse mammary tumors that are in their early egg transfers.

The late mortality rate of eggs inoculated with EAT 1, and an A strain mammary tumor, remained very low for 8 transfers, but thereafter rose rapidly until the 12th transfer, at which point the tumor was lost because the eggs were not harvested soon enough. A contributing factor was the incubator trouble which, by causing an increased number of deaths in the early days of incubation, limited the number of eggs from which transfers could be made.

The late mortality rate of eggs inoculated with EAT 2, our second A strain tumor, remained very low for 20 egg transfers. From this point, however, it increased and now, in its 41st transfer, is such that eggs must be harvested on the 13th or 14th day of incubation in order to continue its further passage.

Cultures showed that only 1.4 per cent of the late mortalities were due to bacterial infection.

2. On the Relation of the Amount of Tumor Tissue Injected to the Lethal Effect of the Tumor

In a special experiment made to investigate this point, the lethal effect of tumors grown from 0.1 cc. of tumor tissue was compared with that of those grown from 0.01 cc.

Between the 35th and 40th transfers of ET, each tumor harvested was diluted to 5 times its volume with a mixture of yolk, amniotic fluid, allantoic fluid, and blood obtained from the chick in which the tumor was grown. Part of this 1-in-5 dilution of tumor tissue was then diluted further to 10 times its volume with the same fluids. By this method a 1-in-5 and a 1-in-50 dilution of each tumor harvested was prepared for inoculation into eggs. Two hundred eggs were each injected with 0.5 cc. of the 1-in-5 dilution and 224 with 0.5 cc. of the 1-in-50 dilution.

All the eggs that died or were harvested between the 10th and 17th days of incubation were examined for tumors. Ninety-nine per cent of those inoculated with the 1-in-5 dilution, and 93 per cent of those with the 1-in-50 dilution had tumors.

After subtracting the number of eggs that did not develop tumors, from the total number inoculated with each dilution, a graph (Fig. 2) was constructed to compare the percentages of late deaths of eggs inoculated with the two dilutions. Allowance was made for the few live eggs that were harvested on the 13th and 14th days to obtain tumor tissue for further transfer of the tumor. Since tissue from each tumor used in this experiment was injected into eggs in two different dilutions, it may be assumed for a basis of comparison between the results obtained from the two dilutions, that the quality of the tumor tissue used was a constant. Further, since the amount of the mixture of fluids from the chick was the same in both dilutions, this factor, too, was a constant. The only variable, then, in the experiment, was the amount of tumor tissue injected. Therefore, the results show that the lethal effect of a tumor grown in a yolk sac depends to some extent on the amount of tumor tissue inoculated.

3. Pathological Findings in Chicks with Serially Transferred Tumors

Gross observations.—These were first made on chicks that died from yolk sac tumors. Postmortem degeneration was often sufficiently advanced to have caused changes, so for most of our gross observations we relied on living chicks from eggs that had been incubated 13 or 14 days and which had been inoculated with ET, EAT 1, or EAT 2 after the tumors had developed increased lethal properties. These chicks were generally smaller than those from non-inoculated eggs incubated for the same time. Petechiae and congested blood vessels were evident in some. Also, in contrast to the control chicks, the livers of the tumor-bearers were apparent through their stretched, translucent abdominal walls.

On opening the tumor-bearing chicks, their livers were found to be enlarged. The color of these livers varied considerably, presenting 3 main types of surface coloration—either a pale or red or partly pale and partly red. Some livers revealed very deep red blotches. The edges and tips of the lobes of all three general types were frequently yellow or gray-yellow, and occasionally showed pin points of red. Some livers, in addition to manifesting yellow or gray-yellow margins, showed patches and streaks of the same color over their surfaces.

The hearts of tumor-bearing chicks were larger than those of the controls. When squeezed with forceps they were much softer and flabbier than normal, with large, red atria.

The developing kidneys of tumor-bearers were only occasionally of a deeper pink than in the controls.

The blood of tumor-bearing chicks was more

watery, and the blood vessels of the membranes were much paler than those of controls.

Microscopic observations.—These were made principally on the tissues and organs of chicks of the type relied on for gross observations.

In the liver the earliest and most consistent abnormality seen was a wide separation of its tubular cords (Figs. 3, lower and 4). Since the reticuloendothelium was adherent to the separated cords, the lesion represented a sinusoidal dilatation rather than an edema. When the condition was general throughout the liver the sinusoids were widely dilated, but when the lesion had a patchy distribution the degree of dilatation was usually not so great. Some dilated sinusoids contained relatively few erythrocytes, in which instance the liver was pale on gross appearance. But others contained more; indeed, some were packed with erythrocytes (Fig. 5). When the sinusoids were well filled with erythrocytes the liver was red upon our gross examination. In some instances large pools of erythrocytes with no liver cords in their midst were seen; these accounted for the deep red blotches occasionally noticed on the surface. Globules of muddy pigment were seen both in reticuloendothelial cells and free in the sinusoids.

In many of the livers, areas near the surface were seen in which sinusoids were packed with erythrocytes that had lost their cytoplasm and whose nuclei showed pyknosis and fragmentation (top of Fig. 4 and Fig. 6). Where the degenerative changes in erythrocytes were not advanced, the adjacent liver parenchyma was healthy; but when the changes in erythrocytes were advanced, the adjacent parenchyma showed degeneration or necrosis. The early change was a separation of the individual cells of the cords, (Fig. 6), the late, a failure of staining (top of Fig. 3). Sometimes, particularly in the older livers, calcium deposits were seen in the necrotic parenchyma. On gross observation, the necrotic areas appeared yellow.

Although the sinusoids containing degenerating or necrotic erythrocytes were usually well filled with them, they were not generally nearly so dilated as the sinusoids in the remainder of the liver. The condition illustrated in Fig. 4, in which the sinusoids are so distended with degenerating and necrotic erythrocytes as to be evident in a low power photograph, is the exception rather than the rule.

In no instance were emboli of tumor cells, or any lesion that resembled a secondary tumor, seen in the liver.

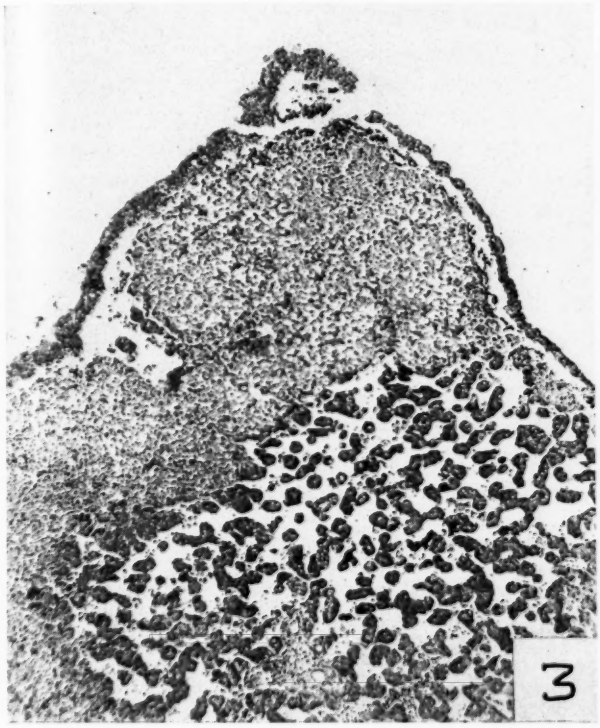
Microscopic investigation revealed no abnormality in the heart muscle or in most of the developing kidneys. In some kidneys, however, there was congestion or even hemorrhage.

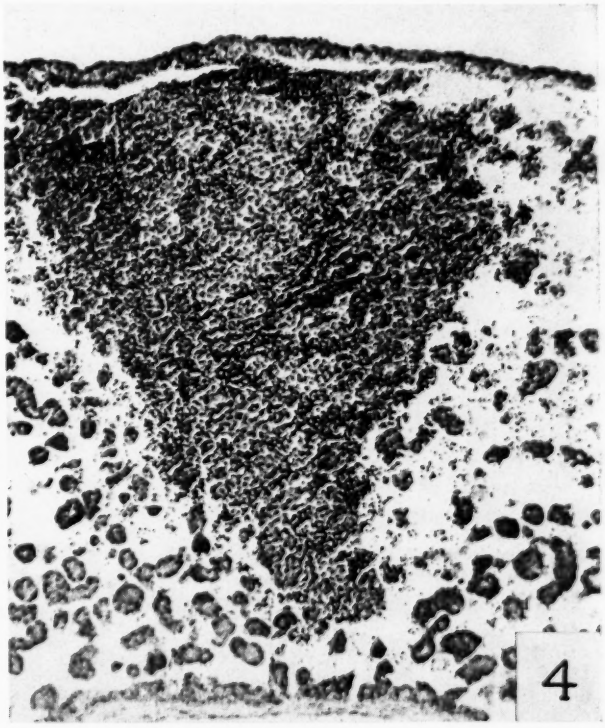
In serial sections cut from skin and subcutaneous tissue demonstrating small hemorrhages the extravasated erythrocytes were usually associated with a developing artery and vein. In some instances the wall of the developing vein appeared deficient enough to allow the escape of erythrocytes into the surrounding soft mesenchyme. Many hemorrhages were distributed along the developing plexus between dermis and subcutaneous tissue.

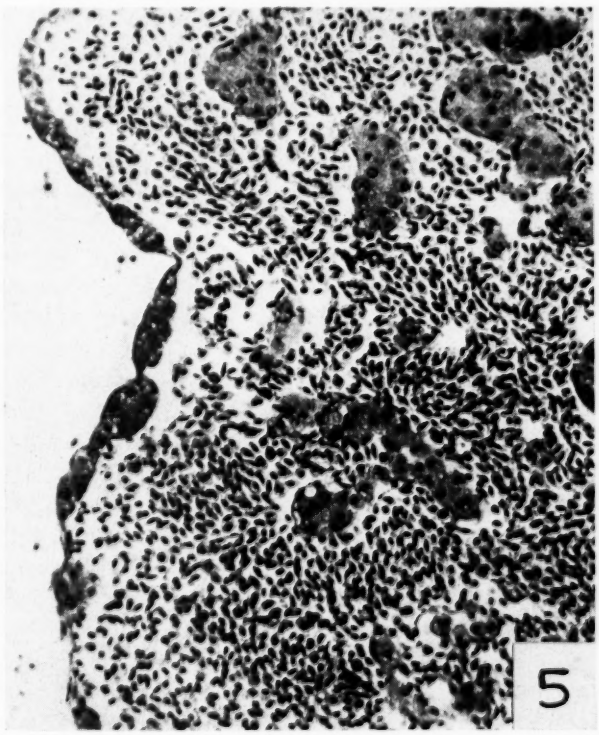
Blood findings.—Blood studies were made on chicks from 13 and 14 day living eggs selected at random that had been routinely inoculated with tumor ET between its 25th and 30th transfers, and on chicks from 16 and 17 day living eggs, also selected at random, that had been routinely inoculated with tumor ECT between its second and fifth transfers. The weights of the tumors contained in the yolk sacs of those chicks, from which blood studies were made, were ascertained. For control studies, noninoculated eggs incubated for the same period were used.

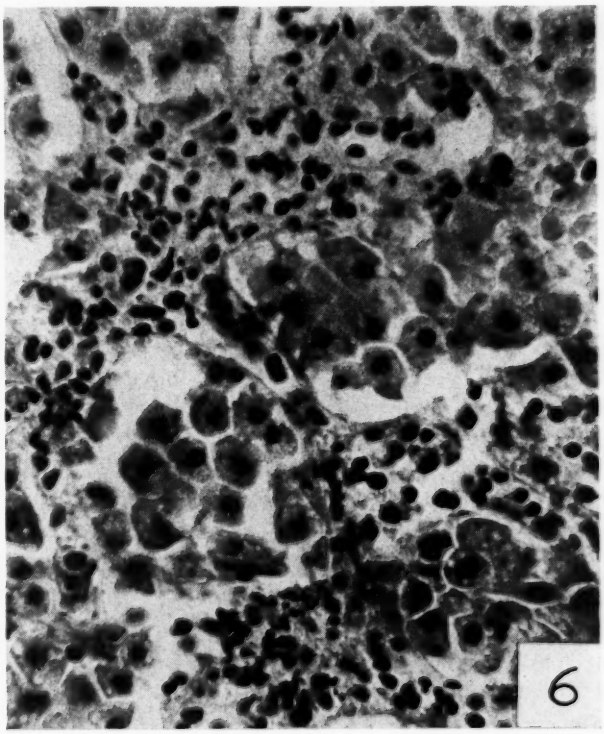
The hemoglobin levels of the 13 and 14 day chicks, whose yolk sacs contained tumors of numerous egg transfers in eggs thereby gaining enhanced lethal properties, are presented in Fig. 7, together with the weights of the tumors recovered. The hemoglobin levels of the 16 and 17 day chicks, whose yolk sacs contained tumors of only a few egg passages and thus had not developed enhanced lethal properties are presented in Fig. 8, together with the weights of the tumor recovered. In neither graph is a positive correlation between tumor weight and the degree of anemia of the chick evident. A comparison of the two graphs, however, reveals that more chicks developed anemia and that it was more severe when the tumor had been transferred many times in eggs.

Although photographs made of the blood of many normal chicks incubated for 13 or 14 days contained a considerable number of erythroblasts, those made from the blood of tumor-bearing anemic chicks of the same age contained, in general, more of them. Furthermore, many of these were of a younger type, and megaloblasts were not uncommon (Fig. 9). The cytoplasm of the erythroblasts in the blood of chicks with severe anemia was extremely deficient in acidophilic material; hence these cells often appeared as blue-tinted ghosts. The younger erythrocytes also were extremely pale. Indeed, old erythrocytes were the only type seen that were well filled with hemoglobin, and not all these were. Large numbers of degenerating cells of the erythrocyte series were also present. Degenerating erythrocytes, how-









DESCRIPTION OF FIGURES 3 TO 6

FIG. 3.—Low-power photomicrograph of section of liver showing marginal necrosis above and dilated sinusoids below. Mag. × 60.

FIG. 4.—Low-power photomicrograph of section of liver showing triangular area of early necrosis containing sinusoids packed with degenerating erythrocytes. Outside this area the sinusoids are widely dilated. Mag. × 100.

FIG. 5.—Medium-power photomicrograph of section of liver showing part of pool of erythrocytes near surface with only a few liver cords within it. Mag. × 200.

FIG. 6.—High-power photomicrograph of section of liver taken through area where parenchymal degeneration is beginning. The sinusoids in this area are packed with degenerating erythrocytes and cells of the parenchymal cords are separating from one another. Mag. × 500.

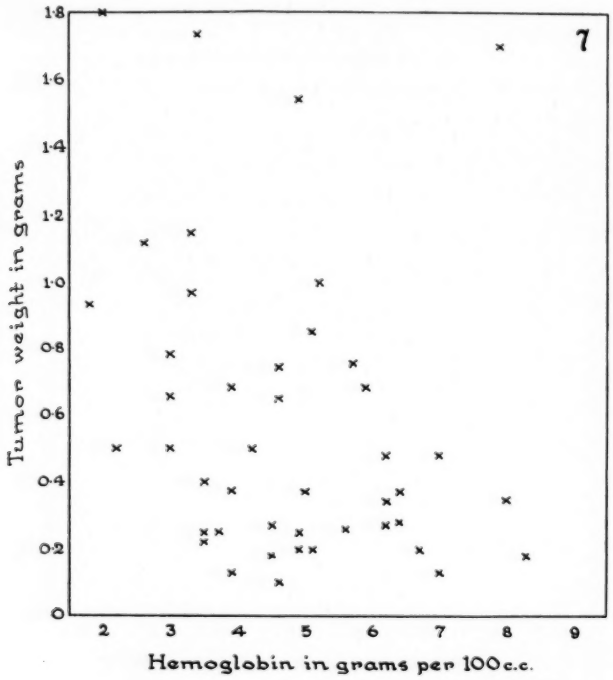


FIG. 7.—Hemoglobin values and tumor weights in 13 and 14 day chicks selected at random whose yolk sacs contained C3H mouse mammary tumors (ET) that had undergone from 25 to 30 transfers in eggs. The hemoglobin value of normal chicks of this age was 7.93± .08.

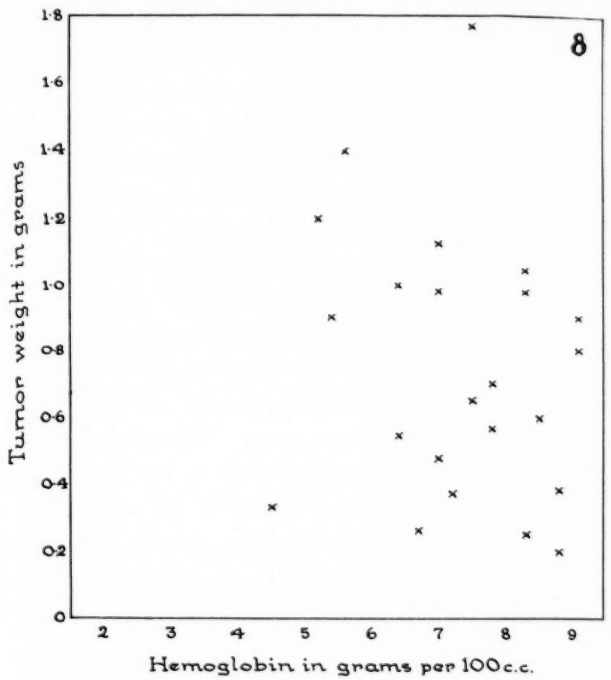


FIG. 8.—Hemoglobin values and tumor weights in 16 and 17 day chicks selected at random whose yolk sacs contained C3H mouse mammary tumors (ECT) that had undergone from 2 to 5 transfers in eggs. Hemoglobin value of normal chicks of this age was 8.86± .21.

ever, were seen in the blood films of normal chicks of this age, but in general they were not so numerous. Only a few granular leukocytes were seen in the films. Lymphocytes are not present in the blood at this stage of development; Sugiyama (12) states that they do not appear until the 17th day. Some thrombocyte counts were made by the indirect method on the blood of both normal and tumor-bearing chicks, the lowest being 1,000, and the highest 24,000 per cu. mm. of blood. Usually the count was somewhat higher in tumor-bearing, anemic chicks than in normal chicks.

Blood sugars.—Preliminary work only has been done on this phase of the investigation. The blood sugar of 8 noninoculated chicks incubated for 14 days ranged from 115 to 140 mgm. per cent. The blood sugar of 8 chicks inoculated with ET at approximately its 40th transfer ranged on the 14th day of incubation from 19 to 108 mgm. per cent but 5 of the 8 chicks had blood sugar levels of 27 mgm. per cent or lower.

DISCUSSION

On whether the continued serial Transfer of mouse tumors in yolk sacs is associated with an increased lethal effect on chicks.—Heilman and Bittner (7) working with mammary tumors from C3H and A strain mice, first described such an effect. Hungate and Snider (8), however, found "no significant increase in the toxicity" of a dba mouse mammary carcinoma that was transferred in eggs 32 times. Their method of inoculation differed from that of Heilman and Bittner and from ours in that the tumor tissue was washed in Tyrode's solution. Less tumor tissue was injected, the extra embryonic coelome route was used for inoculation, and the tumor tissue for serial transfer was selected from the longer lived eggs. Since our dilution experiment showed that less tumor tissue with the same amount of a mixture of fluids from the chicks decreased the lethal effect of the tumor developing on yolk sac inoculation, our results suggest that the failure of Hungate and Snider to observe the same degree of lethal effect seen by Heilman and Bittner and by us, was due more to the small amount of tumor tissue they injected than it was to their washing the tumor tissue in Tyrode's solution to free it from the fluids of the chick. They suggest that "under different experimental conditions an increase in toxicity might result," and indeed in their discussion they note having occasionally observed instances of this phenomenon.

Our findings confirm those of Heilman and Bittner (7). Our experience with the C3H mouse mammary carcinoma seems to have been very much like theirs. We did not, however, observe the very rapid development (on the first few trans-

fers) of an increased lethal effect on cultivating A strain tumors that they did. In our hands two A strain carcinomas developed increased lethal qualities only after 8 and 20 transfers respectively.

We conclude, then, that the serial transfer of mouse mammary tumors in eggs is associated with the development, after a varying number of transfers, of an increased lethal effect on chicks. Moreover our dilution experiment indicates that the increased lethal effect is associated with the tumor cells rather than with the fluids of the chick.

On where and how the lethal effect is exerted.—Our results suggest that the cause of death of chicks bearing tumors with increased lethal properties is to be found in their blood, hearts and livers. The relationship of these lesions to one another will now be discussed.

Taylor, McAfee and Taylor (15) were the first to observe liver lesions in chicks with yolk sac tumors, noting that this organ was pale and blotchy. Kynette, Taylor and Thompson (10) extended these studies and observed abnormal accumulations of erythrocytes, erythrocyte desposits, open frameworks of liver cells separated by spaces, and areas of necrosis in the livers that they examined.

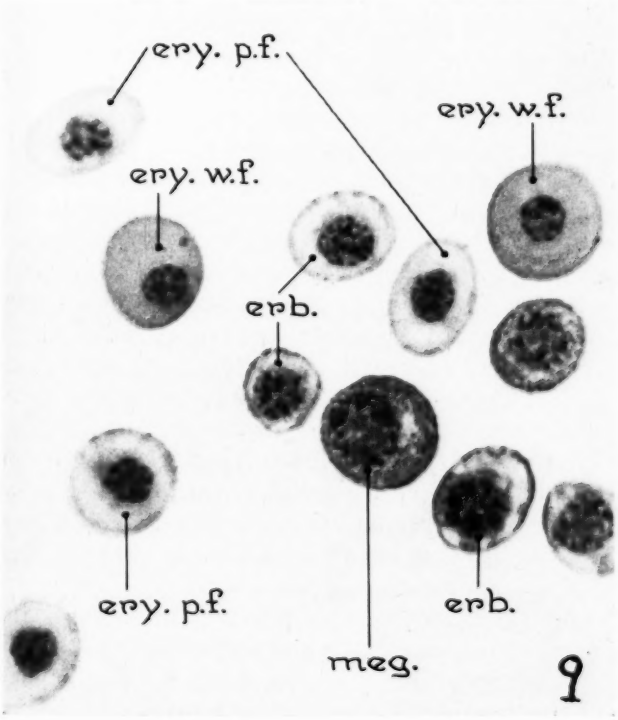


FIG. 9.—Oil-immersion photomicrograph of blood film of chic. with severe hypochromic anemia resulting from yolk sac tumor of many egg passages, showing 2 erythrocytes well filled with hemoglobin(ery.w.f.), 3 erythrocytes containing scarcely any hemoglobin(ery.p.f.), 3 erythroblasts with very little hemoglobin(erb.) and a megaloblast(meg.). Mag. × 1200.

Our observations are in agreement with theirs in these respects. We did not, however, observe any abnormal proliferation of cells bordering sinusoids, a point they stressed.

Twombly and Meisel (17) have also studied the lesions in livers of chicks with yolk sac tumors. They noted that the organ often showed areas of widespread necrosis, but that in less damaged areas "one is apt to come upon areas of blue-staining cells lining the sinusoids." It is their impression that these are cancer cells growing in a nonreacting host without stroma and that their presence accounts for much of the mortality of tumor-inoculated eggs.

Our histological findings offer no support for Twombly and Meisel's thesis. Although they obtained tumor growths in mice inoculated with liver tissue obtained from tumor-bearing chicks, and therefore reasoned that the liver must contain viable tumor cells, we found no histological evidence indicating that the liver lesion is primarily due to either emboli of tumor cells or secondary growths. Liver damage, we think, is to be otherwise explained.

It is known that severe anemia can result in cardiac dilatation and congestive heart failure (18). Since the hearts of the anemic chicks were soft and flabby, it may be assumed that they were affected by the anemia. If the hearts were affected sufficiently to become incompetent, the sinusoidal dilatation observed in the livers of the anemic chicks could be considered to be the result of a congested state induced by the heart's incompetence. In strong support of this contention we found that the extent and degree of dilatation of liver sinusoids bore a rough but generally consistent relationship to the degree of anemia exhibited by the chick. Hence, we conclude that anemia is the cause of an incompetent heart and it in turn is the cause of congested and dilated liver sinusoids.

It might be thought that the degree of hypoglycemia indicated by our preliminary experiments might be a factor in causing the death of the chicks. There is evidence, however, that a degree of hypoglycemia that would cause convulsions in an adult has singularly little effect on the embryo and even on the newborn. Zondek and Wolfsohn (19) have reported finding low blood sugar levels in newborn infants, in some instances obtaining a value of 0 mgm. per cent. In work performed in our own laboratory, as yet unreported, we have found the newborn rabbit to have an extremely low blood sugar level, comparable with those in the tumor-bearing chicks. It does not seem likely, therefore, that the hypoglycemia

of the tumorous chicks would necessarily be a direct cause of their deaths. But nevertheless a sustained hypoglycemia, by making less sugar available for glycolysis in the myocardium, might well assist the anemia in making the heart incompetent.

The degeneration and necrosis of liver parenchyma is not so easy to explain as the dilated sinusoids. Before attempting to do this it is helpful to point out that this lesion occurs when the liver is in a state of active development. Tubular cords of parenchyma are continually budding, growing, and uniting with one another to establish the pattern of the sinusoidal spaces between them. Even under normal conditions it appears that this process achieves only with difficulty an efficient circulatory pathway through the peripheral part of the organ, because small areas of degeneration and necrosis, identical with the larger ones seen in the anemic chicks, were found in this region in some of our controls. With anemia and a less competent heart, it is only to be expected that the oxygenation of the peripheral parts of the liver would be correspondingly less adequate and hence that more degeneration and necrosis would result. It could be expected also that stagnant circulation in these peripheral areas, before they actually become necrotic, could account for the aggregations of erythrocytes seen in the sinusoids either in or about the degenerating or necrotic parenchyma.

We conclude, then, from our studies that anemia is the basic pathological lesion produced by a yolk sac tumor and that the other lesions seen in the chick are consequent to it. We have also shown that the severity of the anemia produced by a yolk sac tumor is much greater after a tumor has been transferred many times than it is when a tumor has been transferred only a few times (compare Figs. 7 and 8). Hence, we conclude further that the increased lethal effect observed when mouse tumors are cultivated serially in eggs represents an increased anematizing effect.

On the nature of the anematizing effect of tumors.—That a malignant growth tends to cause a hypochromic anemia in its host has long been appreciated. Different explanations have been offered for this phenomenon at different stages in the development of knowledge about cancer. These will now be briefly considered in the light of our findings.

First, the older views may be grouped together since they postulate that tumors cause anemia indirectly. They ascribe the anemia variously to hemorrhage, bacterial infection, necrosis of tissue with the absorption of decomposition products, an impaired food intake of the host, or to other conditions brought about by the presence of the tumor. Our finding of severe anemias in chicks with small tumors that evidenced little hemorrhage or necrosis and no bacterial infection does not support the idea that the anemia of malignancy is only an indirect effect of the malignancy.

Second, Taylor and Pollack (16) have suggested that a much closer relationship between malignant growths and anemia exists than has been hitherto suspected. On finding anemia in mice with very early implants and in mice treated with carcinogens even before malignancy developed, they suggested that a cancerous growth or a precancerous condition might have a direct and destructive effect on hemoglobin or inhibit its production. Later Taylor, McAfee and Taylor (15) on finding significant degrees of anemia in chicks with small mouse mammary tumors growing in their yolk sacs stated "it may be presumed that the tumor cells produce a substance or substances which when liberated into the blood stream exert in some manner an inhibitory influence upon the blood hemoglobin concentration." In the same study they found this effect to vary directly with the size of the tumor. In further work with mice Taylor (13) found further evidence to indicate that the anemia varied in relation to the size of the tumor and suggested that each tumor cell makes its contribution toward producing anemia.

Although the theory suggested by Taylor and his co-workers represents in our opinion a very important advance, we do not find it adequate to explain our findings. The concept of a direct effect varying in relation to tumor size does not explain why we should have observed more severe anemias with yolk sac tumors of many serial passages that we did even with larger tumors in their early egg passages. It should be noted in this respect that our study differed from that of Taylor, McAfee and Taylor (15) in that we studied the anemia produced by serially transferred tumors, whereas they studied that produced by first generation egg tumors derived from a standardized transplanted tumor.

Third, Dunning and Reich (2) observed the degree of anemia in the hosts of induced and transplanted sarcomas to be related to the malignancy of the growth. Taylor (13), moreover, also brings this concept into his theory by suggesting that the rate of decrease in hemoglobin concentration with increasing tumor size is related to the degree of malignancy inherent in the tumor concerned.

In order to examine the concept of the anematiz-

ing effect of a tumor being related to its degree of malignancy in the light of our findings, it is necessary to point out first that what we term the anematizing effect probably has functions other than causing anemia. Taylor and Pollack (16) were first to suggest this. Noting that Craig, Bassett and Salter (1) have shown that the cytochrome content of tumor tissue is lowered, and that Greenstein, Jenrette and White (5) demonstrate that the introduction of malignant tissue into an animal greatly decreases liver catalase, and that cytochrome, liver catalase and hemoglobin all have the same hemin prosthetic group, they suggested that an effect on all 3 porphyrin compounds was exerted in the same manner. Greenstein (6) also points out the relationship between liver catalase and hemoglobin and has suggested a tumor activity directed against the synthesis of the hematoporphyrin prosthetic group. Greenstein and coworkers (3, 4) furthermore make the very important observation that the liver catalase lowering effect of tumors is not an effect of rapidly growing cells, embryonic cells, or a combination of the two, for they have shown that the catalase of the liver is not reduced in regenerating livers, in the livers of pregnant animals, or in animals with growing implants of embryonic tissue. It would seem, therefore, that the effect exerted against these porphyrin compounds is not to be related to the physiological properties of rapidly growing or undifferentiated cells; indeed in our work tumors were grown in an embryonic host that abounded in embryonic and rapidly growing cells, yet anemia did not develop unless tumor tissue also was growing in this host. Hence a rapid growth and an undifferentiated state of cells (the chief criteria for estimating a high degree of malignancy in tumors) are not factors which in themselves produce anemia.

That we observed the anematizing effect to become enhanced without a corresponding increase in tumor size suggests to us that a qualitative rather than a quantitative change occurred in it after tumors had been transferred many times in eggs. Since we worked with tumors from mice known to carry the milk influence, and since many viruses have been shown to attain increased virulence on their serial passage in eggs, we question if our results do not suggest that the anematizing effect is not so much in the nature of a true cellular secretion or excretion as it is the property of some agent associated with, and perhaps causing, the tumor process, and of a kind that might be expected to exhibit variation on prolonged serial transfer in eggs.

SUMMARY

The serial cultivation of mammary tumors from C3H and A strain mice in the yolk sacs of hens' eggs resulted in chicks dying in increasing numbers and progressively earlier. The lethal effect of any tumor developing from the inoculation of eggs with tissue from tumors that had been transferred often enough to have gained enhanced lethal properties, was found to depend to some extent on the amount of tumor tissue with which the yolk sacs were inoculated.

The chief pathological findings in the chicks so affected were: hypochromic anemia, enlarged flabby hearts, and enlarged livers, often showing peripheral necrosis. Reasons are given to show that the basic lesion produced by the tumor in the chick is hypochromic anemia and that the other pathological lesions are consequent to it. In view of this and of the fact that the severity of anemia produced by the tumors increased with their serial transfer in eggs, it was concluded that the increased lethal effect of tumors that develops on their serial egg transfer represents an increased anematizing effect.

Since the anematizing effect on the yolk sac tumors we studied was not found to be related to their size, and since they were mammary tumors from strains of mice that carry the milk influence, and since the effect became enhanced on the serial transfer of the tumors, the suggestion is made that the anematizing effect may be more in the nature of some agent associated with the tumor process than a secretion or excretion of the tumor cell proper.

REFERENCES

1. Craig, F. N., Bassett, A. M., and Salter, W. T. Artificial Benignancy of Neoplasm. VI. Observations on the Oxidative Behavior of Tumors, Artificially Benign Tumors, and Homologous Normal Tissues. Cancer Research, **1**:869-882. 1941.
2. Dunning, W. F., and Reich, C. Studies on the Morphology of the Peripheral Blood of Rats. III. Rats with Induced and Transplanted Tumors. Cancer Research, **3**:266-274. 1943.
3. Greenstein, J. P., and Andervont, H. B. Note on the Liver Catalase Activity of Pregnant Mice and of Mice Bearing Growing Embryonic Implants. J. Nat. Cancer Inst., **4**:283-284. 1943-44.
4. Greenstein, J. P., Jenrette, W. V., and White, J. The Relative Activity of Xanthine Dehydrogenase, Catalase, and Amylase in Normal and Cancerous Hepatic Tissues of the Rat. J. Nat. Cancer Inst., **2**:17-22. 1941.
5. Greenstein, J. P., Jenrette, W. V., and White, J. The Liver Catalase Activity of Tumor-Bearing Rats and the Effect of Extirpation of the Tumors. J. Nat. Cancer Inst., **2**:283-291. 1941.
6. Greenstein, J. P. Tumor Enzymology. J. Nat. Cancer Inst., **3**:419-447. 1943.

7. Heilman, F. R., and Bittner, J. J. Observations on Mouse Tumors Cultivated in the Yolk Sac of the Embryonic Chick. Cancer Research, **4**:578-582. 1944.
8. Hungate, R. E., and Snider, H. The Nutritional Adequacy of the Embryonated Egg for Growth of a Mammalian Tumor. University of Texas Publication No. 4507 "Cancer Studies," 53-63. 1945.
9. King, J. T., Ball, Zelda B., and Menefee, E. C. Tumor-Bearing Chicks Hatched from Eggs Inoculated with Mouse Carcinoma. Proc. Soc. Exper Biol. & Med., **57**:3-4. 1944.
10. Kynette, A., Taylor, A., and Thompson, R. C. Effect of Egg Grown Heterologous Tumor Tissue on the Chick Embryo. University of Texas Publication No. 4507 "Cancer Studies." 1945. pp. 65-75.
11. Miller, B. F., and Van Slyke, D. D. A Direct Microtitration Method for Blood Sugar. J. Biol. Chem., **114**:583-595. 1936.
12. Sugiyama, S. Origin of Thrombocytes and of the Different Types of Blood-Cells as Seen in the Living Chick Blastoderm. Contrib. Embryology, Carnegie Inst., Wash., **17-18**:121-145. 1926.
13. Taylor, A. Changes in Hemoglobin Concentration, Total Hemoglobin, and Blood Volume Associated with Tumor Growth. University of Texas Publication No. 4507 "Cancer Studies", 95-102. 1945.
14. Taylor, A., Hungate, R. E., and Taylor, D. R. Yolk Sac Cultivation of Tumors. Cancer Research, **3**:537-541. 1943.
15. Taylor, D. R., McAfee, Marguerite, and Taylor, A. The Effect of Yolk Sac-Cultivated Tumors on the Hemoglobin Level in the Embryonic Chick. Cancer Research, **3**:542-545. 1943.
16. Taylor, A., and Pollack, M. A. Hemoglobin Level and Tumor Growth. Cancer Research, **2**:223-227. 1942.
17. Twombly, G. H., and Meisel, Doris. The Growth of Mammalian Tumors in Fertile Eggs. Cancer Research, **6**:82-91. 1946.
18. White, P. D. Heart Disease. Third Edition, New York: The Macmillan Company. 1944, pp. 537.
19. Zondek, H., and Wolfsohn, Gerda. A Contribution to the Question of the Secretion of the Fetal Islands of Langerhans. Acta Med. Scandinav, **106**:468-478. 1941.

The Use of the Mouse Eye in Transplantation Experiments*

Harry S. N. Greene, M. D.

(From the Departments of Pathology and Surgery, Yale University School of Medicine, New Haven 11, Connecticut)

(Received for publication March 8, 1947)

The anterior chamber of the eye has proven an almost ideal site for tissue transplantation. The technic of transfer is simple, a high percentage of takes is obtained and the growing tissue can be followed by direct visual observation or even subjected to microscopic examination. A further advantage derives from the fact that the chamber supports the growth of heterologous tissues whereas, in other bodily sites, such tissues invariably fail to survive. Moreover, tissues grown in the chamber are usually readily separable from the tissues of the host; they contain little desmoplastic reaction, and in general are excellent material for chemical or immunological study.

In this laboratory, animals such as the guinea pig and rabbit have been used extensively in experiments on transplantation of organs and tissues to the anterior chamber of the eye. The expense of securing and maintaining such animals is considerable in relation to that entailed in obtaining and providing for mice and for this reason, a modification of the technic has been perfected to permit the easy and rapid utilization of mice for the same purpose.

METHOD

The technic used for anterior chamber transfer in larger animals is unsatisfactory when applied to mice. Securing the mouse to an animal board is difficult and time-consuming. A local anesthetic is inadvisable because of the proximity of the conscious animal's teeth to the operative field. The combination of a small eye, a resentful animal and a cumbersome restraining apparatus makes an ordeal of the procedure.

The technic adopted eliminates such difficulties. General anesthesia is effected by suspending the mouse by its tail in a drinking tumbler containing several gauze sponges soaked in ether. Sufficient anesthesia is obtained in 30 seconds, and as this step is carried out by an assistant, the time may be utilized by the operator in placing the tissue to be transferred in a trocar. The trocar is made by shortening the bevel at the tip of a 20-gauge hypodermic needle. A suitable, tight-fitting plunger can be manufactured or obtained simply by selecting a wire stylet of proper size from the stock supplied with the needles and applying a knob of sealing wax or other plastic material to one end.

A minute fragment of tissue is placed in the mouth of the trocar and manipulated into its barrel. This is usually readily accomplished by retracting the plunger to exert suction and prodding the fragment with a fine needle. Sometimes this operation may be irksome, particularly if the tissue is dry or sticky, but caution should be urged against the use of so-called physiological saline in attempt to overcome the difficulty. Stock saline solutions in general use are not physiological and are often sufficiently toxic to cause death of the tissue.

The anesthesia rarely lasts longer than a minute and subsequent procedures must be executed rapidly. The mouse is held loosely in the left hand and the lids of its right eye forced apart with the thumb and index finger. Slight pressure with these fingers causes the eye to protrude sufficiently to allow adequate exposure for the operation. The anterior chamber is opened close to the upper border of the corneo-scleral junction by means of a short, quick jab with a double-edged corneal knife. The knife is of a size generally used in ophthalmological work and is readily secured in any instrument house. Single-edged knives or pointed Bard-Parker blades are not satisfactory for they result in a triangular incision through which the iris may herniate. The incision is made only of sufficient length to admit the trocar and must be accomplished entirely by the downward thrust of the knife for the instability of the protruding eye prohibits side cutting. The point of the knife should be directed slightly forward in making the incision in order to enter the chamber without cutting the iris.

The trocar, held between the thumb and middle finger of the right hand, is inserted into the chamber through the incision and the fragment expressed by pushing the plunger with the index finger. In order to prevent extrusion and escape of the fragment through the excision, all pressure exerted on

*This investigation was aided by grants from The Jane Coffin Childs Memorial Fund for Medical Research, The Donner Foundation, and the David, Josephine and Winfield Baird Foundation, Inc.

the animal by the left hand should be released before withdrawal of the trocar. The fragment now free in the chamber, is forced into a wedged position at the inferior angle of the iris by applying light pressure along the corneal surface with a blunt instrument. The incision is not closed.

Considerable trouble may be encountered in preventing the escape of soft, slippery tissues such as embryonic brain when the trocar is withdrawn. This difficulty may be circumvented by incising the iris as well as the cornea at the limbus and directing the trocar behind the superior half of the iris, through the pupil and into the inferior portion of the anterior chamber. With withdrawal of the trocar, the fragment is almost invariably caught at the pupillary border and retained in the chamber.

The whole operation can be performed rapidly after short practice. The average speed in this laboratory is 2 mice a minute and further acceleration may be obtained if 2 anesthetists are employed. However, it should be emphasized that sterility is essential; instruments should be boiled and aseptic technic maintained throughout the procedure. The mouse eye is apparently much more susceptible to infection than is the eye of the guinea pig or rabbit.

RESULTS

The mouse eye has been used extensively in this laboratory for the past 3 or 4 years, particularly in the homologous and heterologous transplantation of cancer and embryonic tissue. The results have been most satisfactory in experiments in which only small growths of tissue were desired. The anterior chamber of the mouse eye is not large enough to contain growths of the size needed for

FIG. 1.—Mouse of strain A bearing anterior chamber transplant of embryonic mouse tongue treated with methylcholanthrene. This photograph was taken 83 days after transfer. Histologically, the tumor was an epidermoid carcinoma.

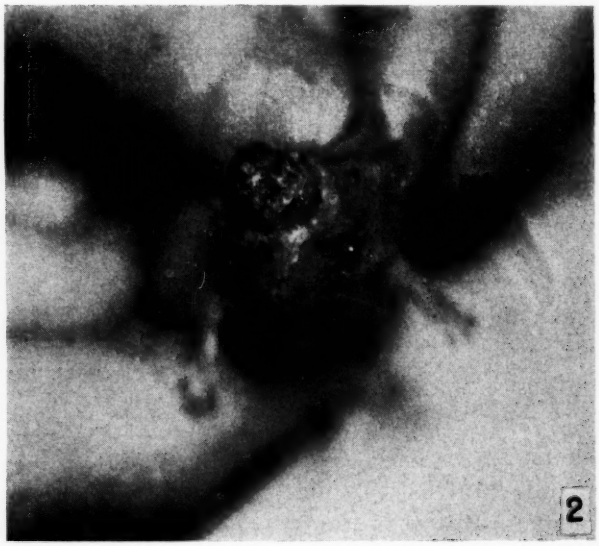


FIG. 2.—Mouse of C3H strain bearing anterior chamber transplant of Brown-Pearce rabbit tumor. This photograph was taken 140 days after transfer and represents a recurrence of growth after partial regression of transplant. Histological sections at death were identical with those of same tumor grown in rabbit.

DESCRIPTION OF FIGURES 3 TO 8

FIG. 3.—Section of eye of C57 mouse bearing a transplant of a mouse ovarian embryoma. The animal was killed 2½ weeks after transfer. Mag. × 35.

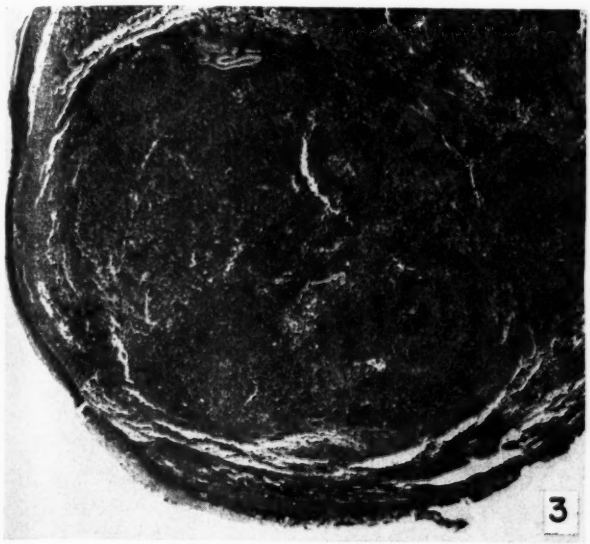
FIG. 4.—Higher power view of previous section. Mag. × 320.

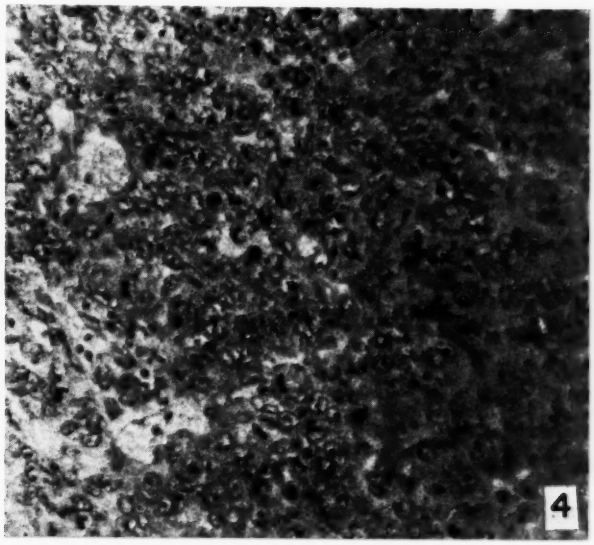
FIG. 5.—Section of eye of Swiss mouse bearing transplant of mammary carcinoma originating in CBA mouse. Attempts to transfer tumor from CBA mouse to subcutaneous tissues of Swiss mice were unsuccessful but high percentage of takes was obtained when tumor tissue from Swiss eyes was transferred to subcutaneous spaces of other Swiss mice. Mag. × 50.

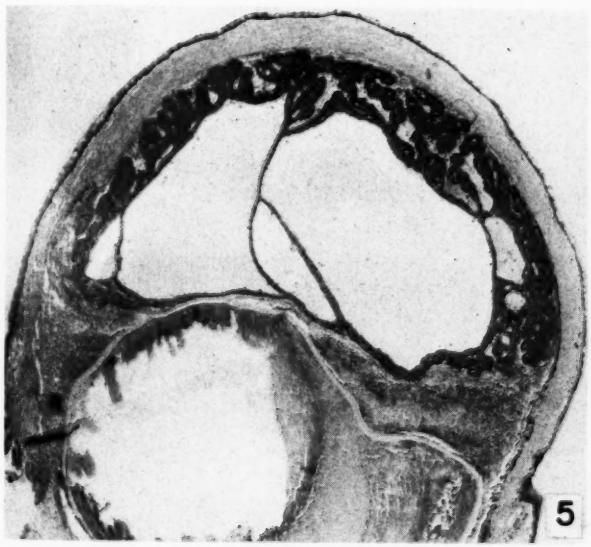
FIG. 6.—Higher power view of previous section. Mag. × 350.

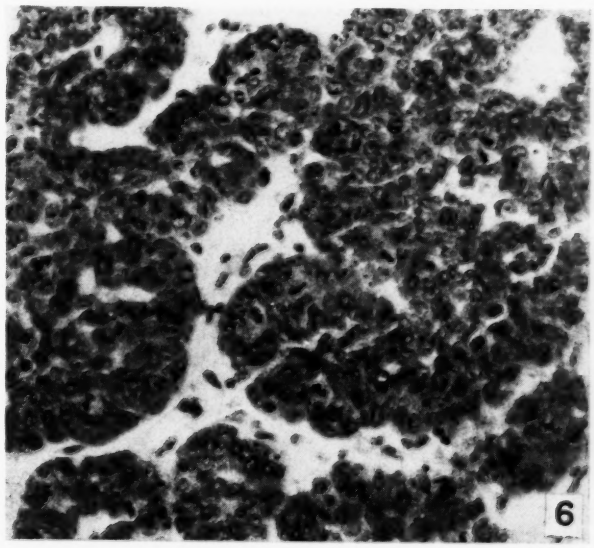
FIG. 7.—Section of eye of Swiss mouse bearing a transplant of Cloudman melanoma. As in previous case, transfer from the eye to subcutaneous space resulted in takes whereas direct subcutaneous transfer from parent strain to Swiss was unsuccessful. Mag. × 50.

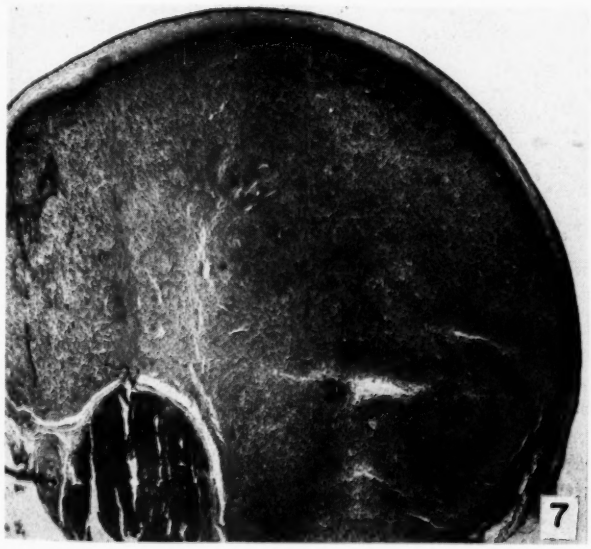
FIG. 8.—Higher power view of previous section. Mag. × 225.

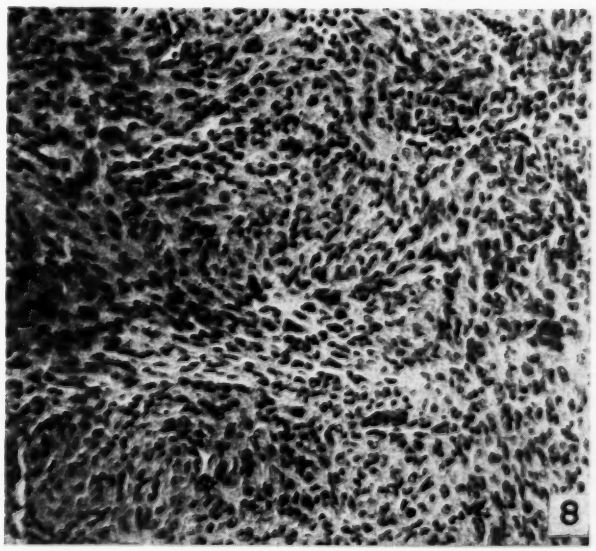












FIGS. 3-8

chemical or immunological studies and other species are more suitable for such purposes. Large growths do occur in the mouse eye but they are necessarily associated with rupture of the cornea and external protrusion. Infection is always present in such cases and the tissue is valueless for further passage or other experimentation.

Homologous transfers.—The transfer of mouse tumors to the anterior chambers of eyes of other mice results in a high percentage of takes and rapid growth. The age or sex of the recipient has played no observable part in the behavior of the graft in our series of transfers. Moreover, the so-called influence of strain appears to be largely negated when the anterior chamber is used.

Reports in the literature suggest that many mouse tumors are strain-specific. This has not been the case with the great majority studied in this laboratory. It is true that, in early stages of development, mouse tumors, like tumors in other animal species, are dependent in nature and are transplantable only autologously or to other animals of the same strain. But, with continued development in the original host, they attain autonomy and the ability to survive and to grow on homologous and heterologous transfer. Thus, only autologous takes or takes in animals of the same strain may result from the transfer of tissue obtained at biopsy whereas growth in foreign strains and even in alien species occurs with material derived from autopsy, 1 to 3 months later. It is suggested that the standard strain-specific mouse tumors, well known in all cancer research laboratories, were originally transferred from a spontaneous growth before autonomy had been attained.

In any case, it has been our experience that, if the primary host survives a sufficient period of time after the origin of the tumor, the growth loses its "strain specificity" and becomes transplantable in many strains. At the same time a variation persists in the ease with which the autonomous tumor is transferred to various strains. Thus, one tumor originating in a C3H mouse could be transferred directly to dba mice but not to C57 mice. On the other hand, when the same C3H tumor grown in a dba mouse, was used, a high percentage of takes occurred in C57 mice. Such results suggested that the strain of the donor exerted an influence on the outcome of transfer and it was felt, for many reasons, that this might be referable to an antagonistic interaction between the connective tissues of the intended recipient and the tumor stroma carried along with the parenchyma at transfer (1).

The point to be emphasized in the present connection bears on this suggestion, for takes occur on direct transfer from the primary host to the eye of the new strain in cases in which successful subcutaneous transplantation requires the intermediation of another host. After growth in the eye, the tumor can be readily transferred to the subcutaneous space of the new strain and carried by serial passage in that site. If, as suggested, incompatibility reactions between the connective tissues of the donor and recipient form the basis of the failure of primary subcutaneous transfer, then it must be assumed that such reactions do not occur in the anterior chamber. Other experiments substantiate this assumption and offer a plausible explanation. Histological study of anterior chamber grafts removed at short intervals after transfer shows that the transplanted stroma dies before vascularization by the new host begins while, in the interim, the parenchyma proliferates in the manner of a tissue culture. Thus, when vascularization eventually occurs and the connective tissues of the new host are brought in contact with the implant, the old stroma has largely disappeared and the basis for any serious interaction has been removed.

Growth is rapid in the anterior chamber so that after several weeks, the cornea ruptures and the tumor protrudes as a fungating mass. Large portions become necrotic, covered with dried crust and may eventually slough off. In such cases, the animal may live long enough for metastasis to occur, but usually the tumor becomes infected

DESCRIPTION OF FIGURES 9 TO 14

FIG. 9.—Section of eye of C57 mouse bearing transplant of the upper third of 2 mm. C3H embryo. Section was taken 80 days after transfer and shows growth of cartilage and squamous epithelium. Mag. × 50.

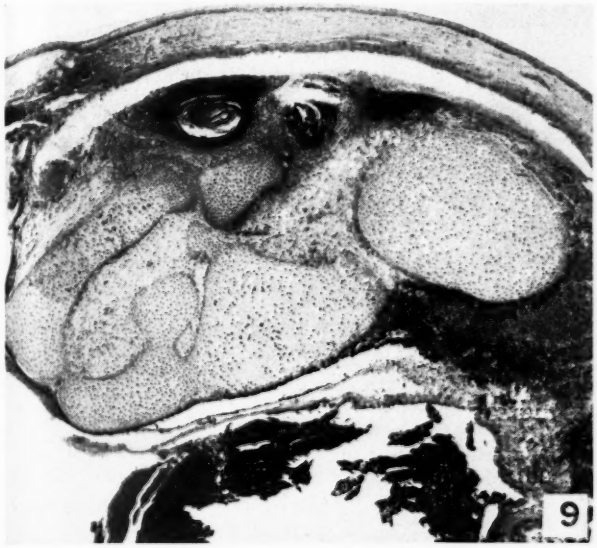
FIG. 10.—Section of eye of C57 mouse bearing transplant of intestine from C57 embryo. Section was taken 56 days after transfer. Mag. × 35.

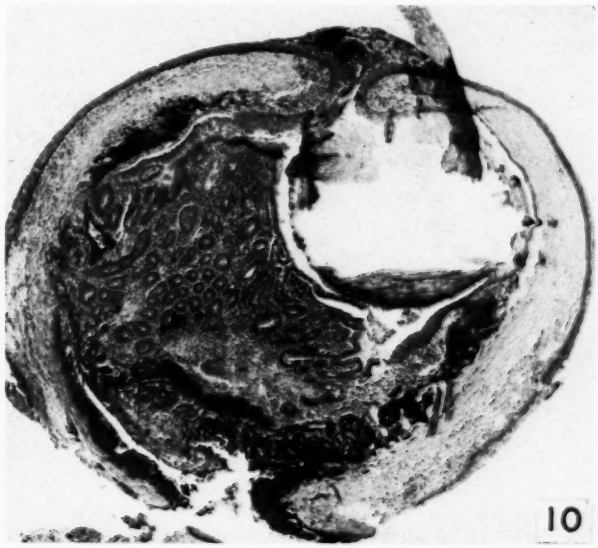
FIG. 11.—Section of eye of Swiss mouse bearing transplant of lung from Swiss embryo. Section was taken at 94 days and shows an epithelialization of alveoli comparable to that seen in so-called lung adenomas of A strain. Mag- × 150.

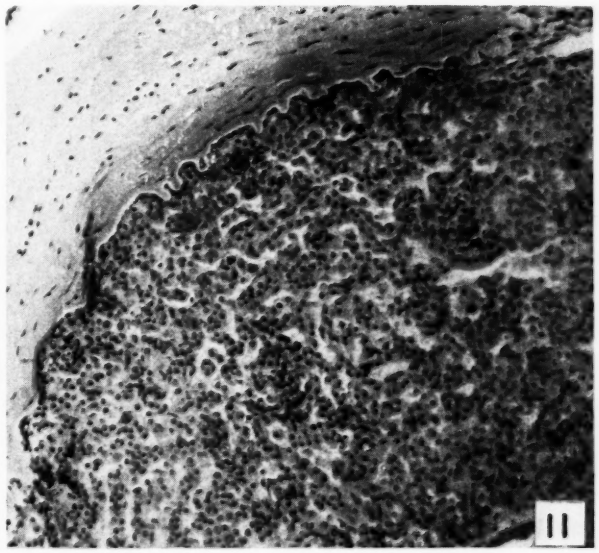
FIG. 12.—Section of eye of Bagg albino mouse bearing transplant of brain from strain A embryo. Section was taken at 39 days and shows growth of ganglion cells as well as of glial elements. Mag. × 300.

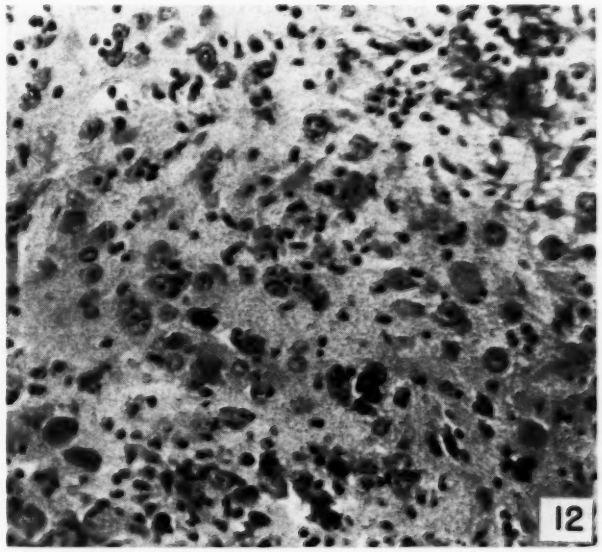
FIG. 13.—Section of eye of strain A mouse bearing a transplant of spleen from strain A embryo. Section taken 390 days after transfer. Mag. × 70.

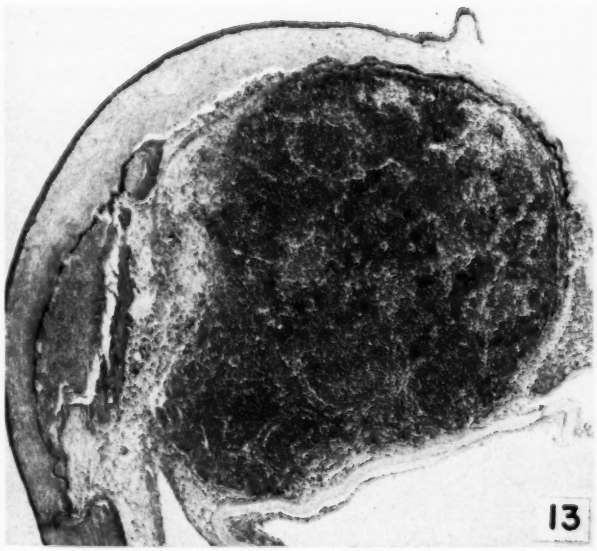
FIG. 14.—Higher power view of previous section. Note megakaryocytes. Mag. × 500.

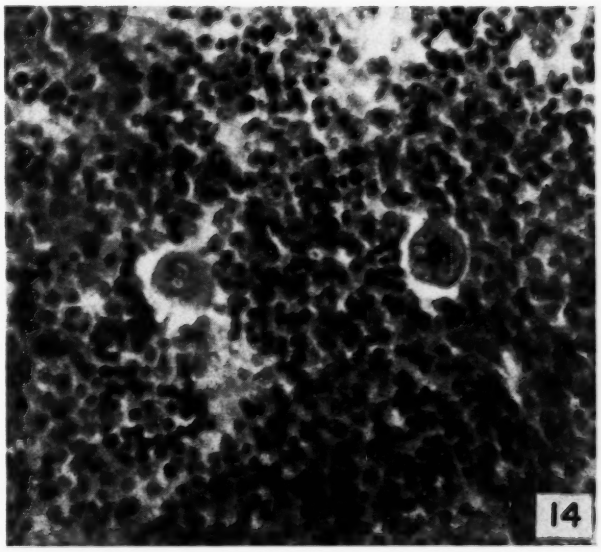












FIGS. 9-14

and death follows without lymphatic extension or dissemination (Figs. 3 to 8).

The transplantation of embryonic tissue is also readily performed in the mouse eye and at the present time, all of the various organs and tissues of the mouse embryo, with the sole exception of the liver, have been successfully transferred to this site. The anterior chamber possesses an advantage in the transfer of small organs as the gonads, spleen and esophagus in that they remain in view and are not lost during early growth phases, as is the case in the expanse of the subcutaneous space. In general, the subcutaneous space is a better nidus for larger organs such as the stomach or lung for it allows their full expansion, whereas in the chamber normal contours and relationships are soon lost and continued growth may lead even to corneal rupture.

A high incidence of takes is obtained and failure appears to depend entirely on infection or faults in technic. The transplanted organ usually reaches its maximum size in from 2 to 3 weeks and no further increase in size occurs. To date, mice bearing organ transplants have been held under observation for as long as 18 months without sign of regression and there is no reason to believe that the grafts will not persist throughout life.

Sections of the transplants obtained several weeks or months after transfer show well developed organs (Figs. 9 to 18). Modifications in differentiation sometimes occur, notably in the lung where squamous metaplasia of bronchial epithelium and epithelialization of alveoli (so-called lung adenomas) are most common.

In several experiments, fragments of adult organs have been used for transfer but potentialities in this direction have not been adequately explored. The fragments survive and actually show some increase in size. Histologically, the structure of the parent organ is duplicated and organization appears normal (Fig. 19). Transplants of adult nerve are an exception in this respect and the aberration is of some interest. The fragments grow rapidly to fill the chamber and, on section, show a disorganized proliferation of Schwann cells bearing a distinct resemblance to tumors derived from these elements (Fig. 20).

The susceptibility of embryonic tissues to the action of carcinogenic chemicals has been reported (2, 3, 5, 6) and the anterior chamber of the mouse eye has been investigated as a nidus for carcinogenesis of this type (Figs. 1, 21 to 24). The technic is essentially identical with that employed in the transplantation of normal embryonic tissues, the only alteration being the addition of a minute crystal of methylcholanthrene to the fragment before transfer. As a rule, an interval of 60 or more days is required before the occurrence of indicative morphological changes, a period of approximately twice the duration required in the subcutaneous space. However, there are distinct advantages, apart from the opportunity for direct visual observation, associated with the use of the eye in such experiments. The eye affords a better medium for the growth of epithelium than does the subcutaneous space, takes occur with higher frequency and structures are reproduced with greater fidelity. Moreover, the connective tissues of the eye are more resistant to the action of carcinogenic chemicals, and sarcomatous growths of the host which might be interpreted as arising in the transplant are far less common.

Heterologous transfers.—Tumors of human and rabbit origin have been successfully transplanted to mouse eyes, although a considerable variation exists in the ease with which the two types of transfer are effected. Transfer from man directly to the mouse gives rise to relatively few takes and much better results are obtained if the tumor is first passed through a guinea pig generation. In contrast, the mouse is a better host for rabbit tumors than is the guinea pig.

The species studied may be classified with respect to the ease of heterotransplantability. A curious relationship was revealed: The mouse and the rabbit constitute one group and man and the guinea pig the other. Transfer within these groups is comparatively easy while transfer between the groups is difficult and attended with a much smaller percentage of takes. It is obvious that

DESCRIPTION OF FIGURES 15 TO 20

FIG. 15.—Section of eye of Strain A mouse bearing a transplant of kidney from a Strain A embryo. Section taken 28 days after transfer. Mag. × 35.

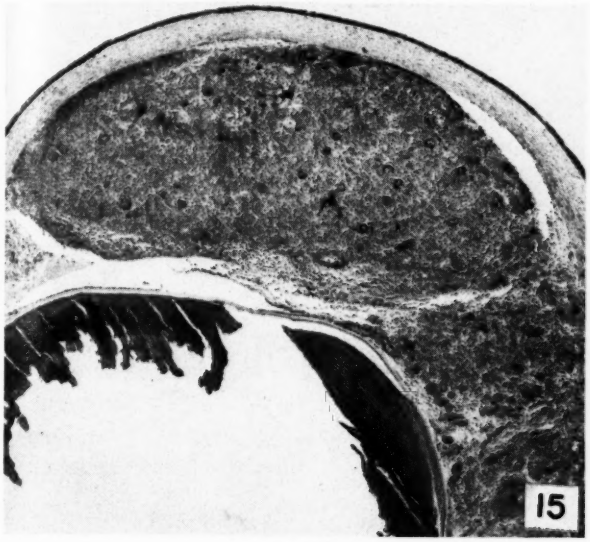
FIG. 16.—Higher power view of previous section. Note persistence of embryonic type glomeruli. Mag. × 300.

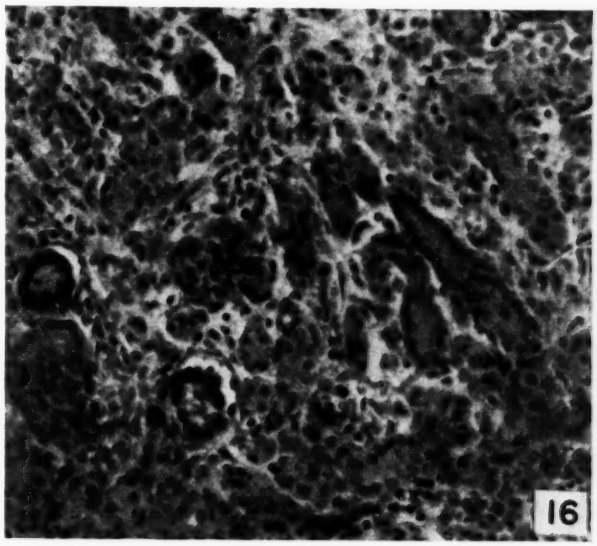
FIG. 17.—Section of eye of ZBC mouse bearing a transplant of testicle from C3H embryo. Section was taken 38 days after transfer and shows growth of epididymis as well as of testicle. Mag. × 30.

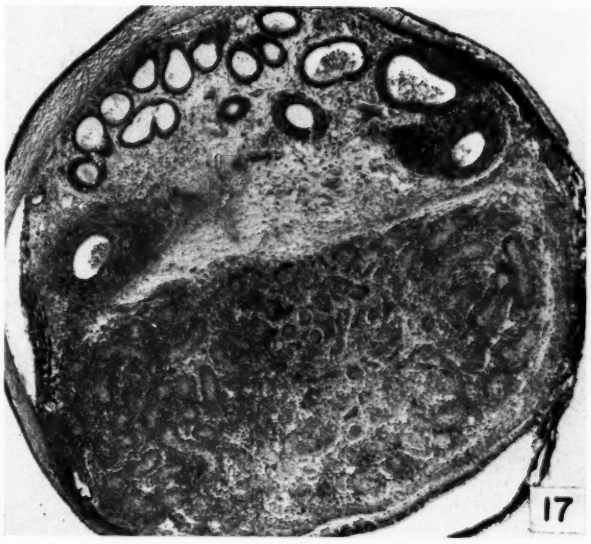
FIG. 18.—Higher power view of previous section. Mag × 225.

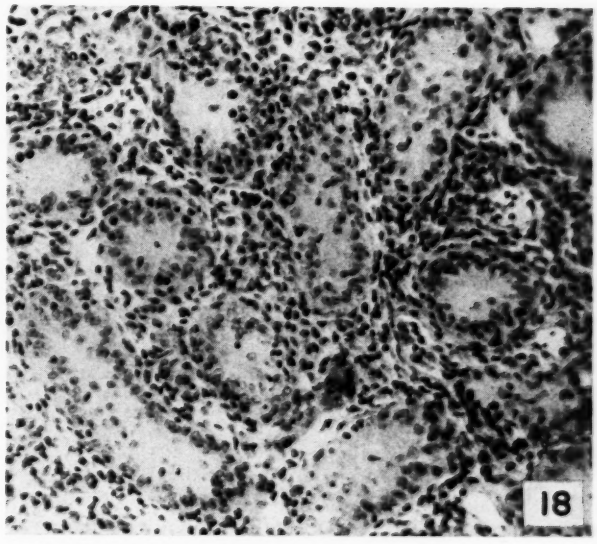
FIG. 19.—Section of eye of C3H mouse bearing transplant of ovary from another adult C3H mouse. Mag. × 85.

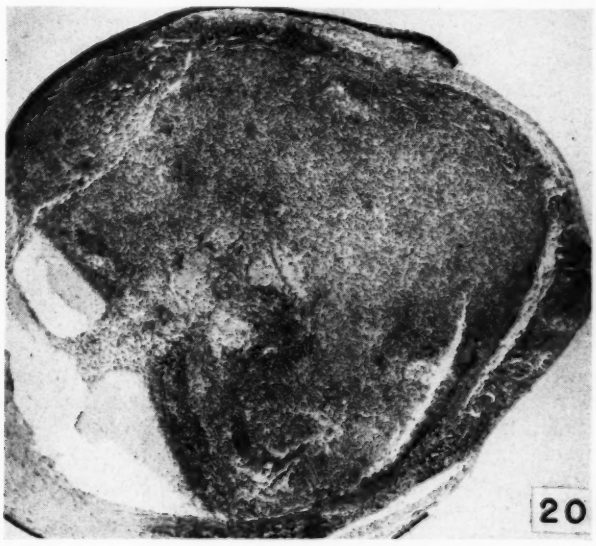
FIG. 20.—Section of eye of Swiss mouse bearing a transplant of sciatic nerve from another adult Swiss mouse. Proliferation of cells resembles a neurofibroma of Antoni type. Mag. × 50.











FIGS. 15-20

this grouping of species also represents a division with reference to the ability to synthesize vitamin C. The relationship may be purely coincidental but metabolic differences between tumor and host tissues presumably occur and might well account for the observed variations. Pertinent investigations are in progress.

The heterologous tumors most fully studied in the mouse have been a human fibrosarcoma (4) (Figs. 25, 26) and the Brown-Pearce rabbit tumor (Fig. 2). Both give rise to a high percentage of takes, grow to fill the eye and are easily carried by serial transfer if the donor mice are killed before the expanding tumor ruptures the cornea.

The only noteworthy alteration in the growth of the fibrosarcoma in the mouse is a tendency of its cells to round off and assume an epithelioid character in sharp contrast to the obvious fibroblastic nature of the tumor in the guinea pig and in man. Curiously, the same alteration occurs on transfer of this growth to the rabbit. The histological appearance of the Brown-Pearce tumor does not change in the mouse (Figs. 27, 28). A peculiarity of its behavior in this species is the frequency of regression and recurrence. The tumor may grow to form a fungating mass as large as the mouse's head, then undergo regression so complete that no trace of tumor can be found in the atrophied eye. However, recurrence is the rule and in several instances renewed growth was not evident until after the lapse of 6 months.

The mouse eye also affords good growth for the embryonic tissues of other species (Figs. 29 to 32). The same species relationships observed in the heterologous transplantation of tumors holds here but is less pronounced and it is much easier to grow human embryonic tissue in the mouse than it is to grow human cancer. The tissues undergo differentiation and organization and, despite the distinct environmental difference, little variation from normal intrauterine development can be found.

DISCUSSION

The object of the present paper was to point out the potentialities of the mouse eye as a transplantation site. The experiments cited and the results obtained require further consideration in relation to the special fields to which they pertain but such discussion is not essential to the immediate purposes of this report and will be presented in later papers describing the experiments in more detail. Sufficient evidence has been offered to justify the conclusion that the mouse eye is a good transplantation site offering a better approach to special problems than other bodily regions and deserving more widespread use than is at present accorded.

SUMMARY

A simple technic of anterior chamber transfer in the mouse has been devised. The technic has been successfully applied to the homologous and heterologous transplantation of tumors and of embryonic tissue, the homologous transfer of adult tissues and the production of carcinomas in transplanted embryonic organs. Illustrative experiments are described.

REFERENCES

1. Greene, H. S. N. The Heterologous Transplantation of Mouse Tumors Induced *in Vitro*. Cancer Research, **6**:396-402. 1946.
2. Greene, H. S. N. The Heterologous Transplantation of Embryonic Mammalian Tissues. Cancer Research, **3**:809-822. 1943.
3. Greene, H. S. N. The Production of Carcinoma and Sarcoma in Transplanted Embryonic Tissue. Science, **101**:644-645. 1945.
4. Greene, H. S. N. The Heterologous Transplantation of a Human Fibrosarcoma. Cancer Research, **2**: 649-654. 1942.

DESCRIPTION OF FIGURES 21 TO 26

Fig. 21.—Section of eye of strain A mouse bearing a transplant of esophagus from strain A embryo. A crystal of methylcholanthrene was added to esophagus before transfer. Section was taken 69 days after transfer and shows epidermoid carcinoma developing from esophageal mucosa. The excessive keratinization appears to be characteristic of epidermoid carcinomas of the mouse produced in this manner. Mag. × 35.

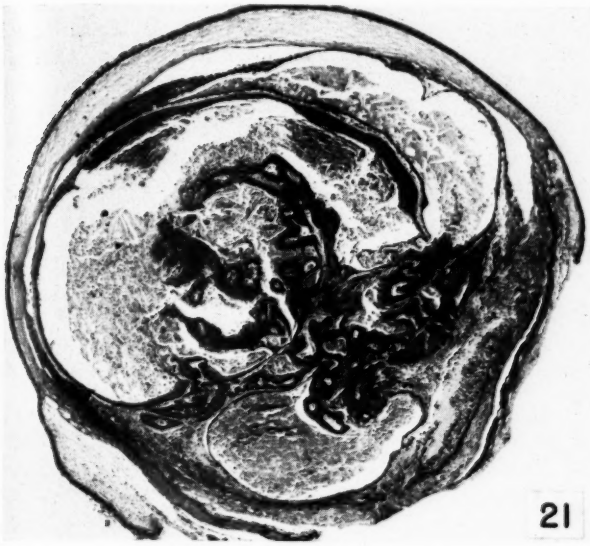
Fig. 22.—Higher power view of previous section. Mag. × 225.

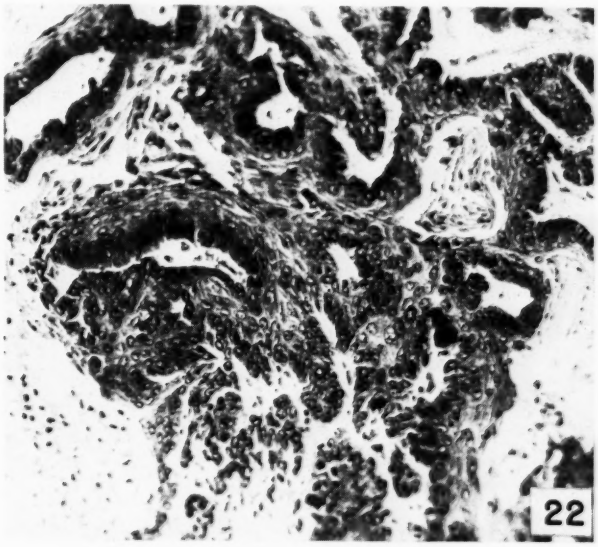
Fig. 23.—Section of eye of C3H mouse bearing a transplant of forestomach from a strain A embryo. A crystal of methylcholanthrene was added to the fragment before transfer and the animal was killed 55 days afterwards. The stomach with its lumen filled with keratin occupies the expanded anterior chamber and small epidermoid carcinoma is arising at one pole. Mag. × 50.

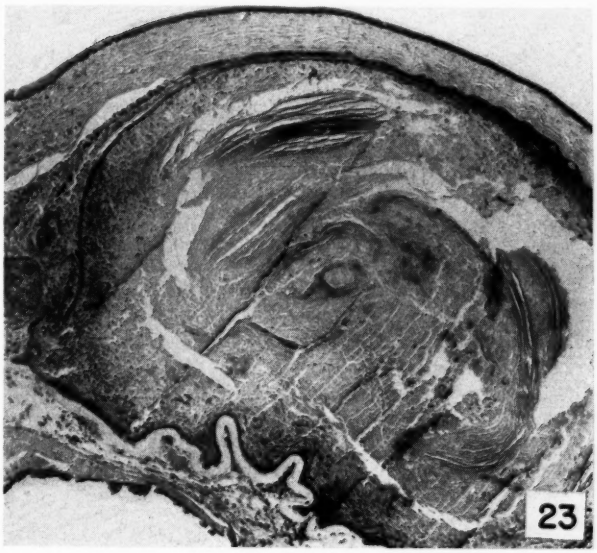
Fig. 24.—Section of eye of C3H mouse bearing transplant of forestomach from a ZBC embryo. A crystal of methylcholanthrene was added to the fragment before transfer and animal was killed 98 days afterwards. Section shows an invading epidermoid carcinoma with much less keratinization than is usually observed. Mag. × 85.

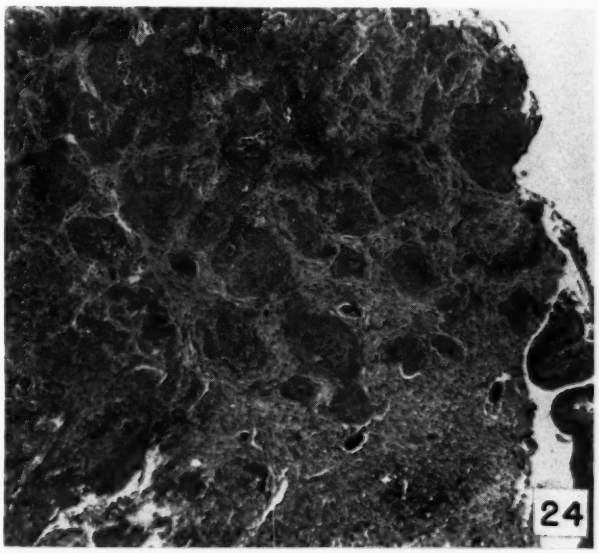
Fig. 25.—Section of eye of C57 mouse bearing a transplant of a human fibrosarcoma. Mag. × 50.

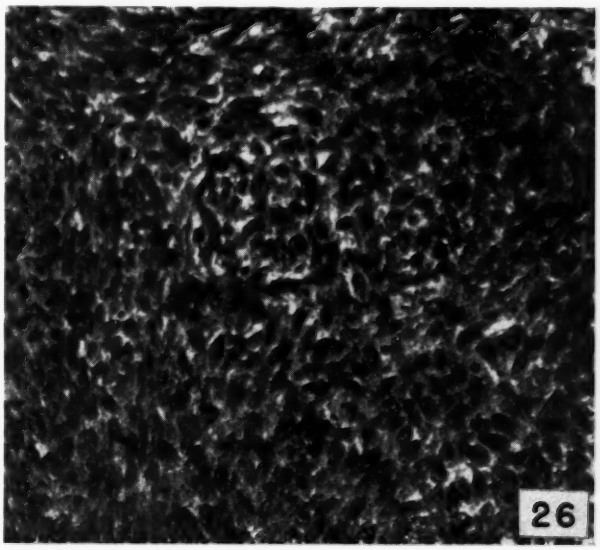
Fig. 26.—Higher power view of previous section. Mag. × 300.











FIGS. 21-26

5. Rous, P., and Smith, W. E. The Neoplastic Potentialities of Mouse Embryo Tissues. I. The Findings with Skin of C strain Embryos Transplanted to Adult Animals. J. Exper. Med., **81**:597-620. 1945.

6. Smith, W. E., and Rous, P. The Neoplastic Potentialities of Mouse Embryo Tissues. II. Contributory Experiments. Results with the Skin of C_3H and Webster-Swiss Embryos; General Considerations. J. Exper. Med., **81**:621-646. 1945.

DESCRIPTION OF FIGURES 27 TO 32

Fig. 27.—Section of eye of strain A mouse bearing transplant of Brown-Pearce rabbit tumor. Animal was killed 10 days after transfer. Mag. × 60.

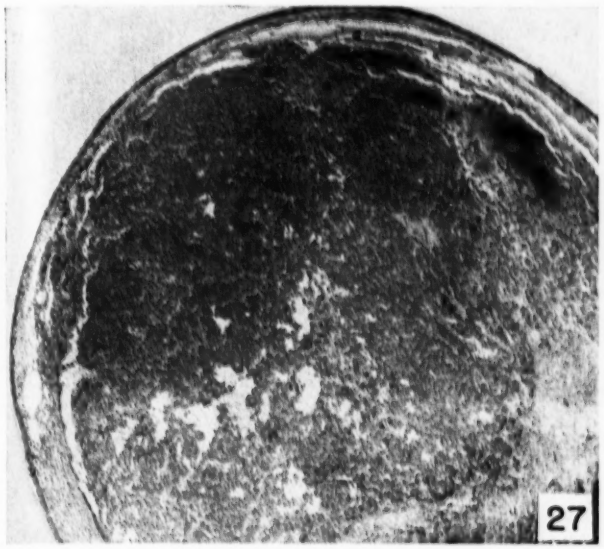
Fig. 28.—Higher power view of previous section. Mag. × 300.

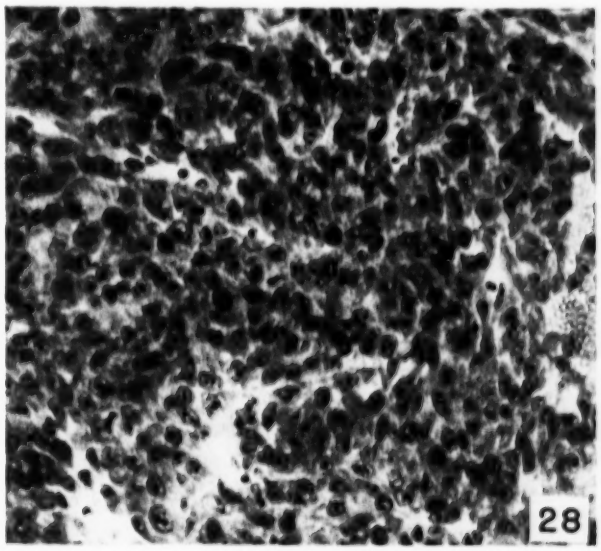
Fig. 29.—Section of eye of Swiss mouse bearing transplant of embryonic human lung. The animal was killed 40 days after transfer. Mag. × 60.

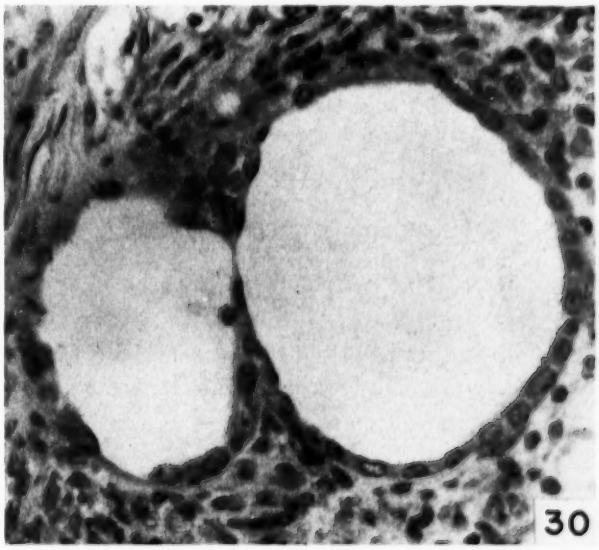
Fig. 30.—Higher power view of previous section to show contiguous bronchial buds. Mag. × 500.

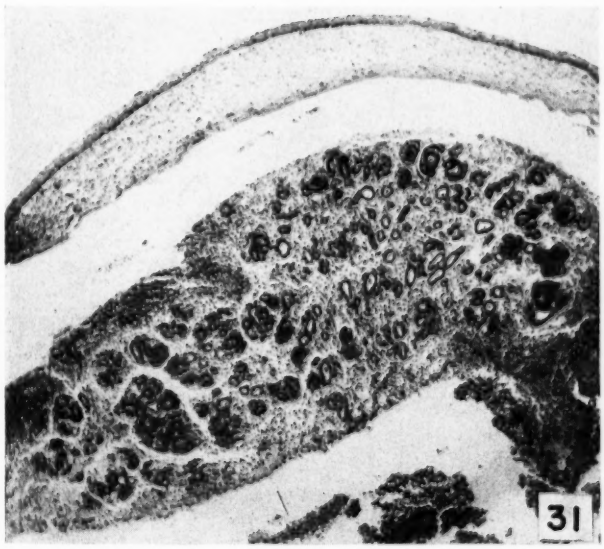
Fig. 31.—Section of eye of Bagg albino mouse bearing a transplant of kidney from guinea pig embryo. Animal was killed 43 days after transfer. Mag. × 50.

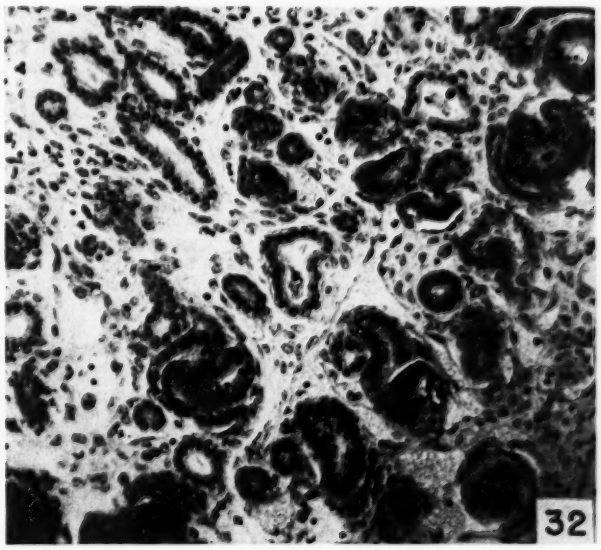
Fig. 32.—Higher power view of previous section. Mag. × 225.











FIGS. 27-32

Carcinoma of the Vaginal Wall in the Rabbit*

Harry S. N. Greene, M. D., B. L. Newton, M. D.† and Albert A. Fisk, B. A.††

(From the Departments of Pathology and Surgery, Yale University School of Medicine, New Haven 11, Connecticut)

(Received for publication March 8, 1947)

The characteristic distribution of uterine cancer in man with a high incidence in the cervix and a low frequency in the fundus does not obtain in the rabbit. Carcinoma of the uterine fundus is by far the most common of all neoplasms in the rabbit (1) whereas cervical cancer has not been observed in our laboratory nor has its occurrence been reported in the literature. The absence of a squamocolumnar junction in the rabbit's cervix suggests an anatomical basis for the species variation. Unlike the situation in man where columnar and squamous epithelium meet in the region of the external os, the columnar epithelium of the rabbit's fundus continues uninterruptedly over the cervix and down the vaginal wall to a junction with squamous epithelium at about the level of the urethral meatus. The object of the present paper is to report three cases of epidermoid cancer at this site. The occurrence of cancer at the squamocolumnar junction in both man and the rabbit despite its different location in the two species emphasizes the significance of the junction as a predisposing factor in carcinogenesis.

MATERIALS AND METHODS

The organization and management of the colony of rabbits in which the tumors occurred have been described in detail elsewhere (2). It should be noted, however, that the colony is maintained in active breeding service, the pedigrees and life histories of all animals are known and all abnormalities in behavior or general health are investigated. From September, 1931 to February, 1947, the extent of the present report, the population was made up of 14 pure breeds, including the Belgian, Beveran, Chinchilla, Dutch, English, Havana, Himalayan, Polish, Rex, Sable and Silver Marten, Siamese Sable, French Silver and Tan breeds, and numerous hybrid lines.

The animals of this colony are subjected to weekly examination with particular attention directed toward a search for neoplastic foci. When tumors are found, biopsies are performed at frequent intervals and the natural history of the growth is followed to its termination. In none of the present cases, however, was the presence of the vaginal tumor discovered during life. In all three instances, the animals bore adenocarcinomas of the uterine fundus and had been under close observation supplemented by frequent laparotomy during the year preceding death. Despite such study, no clinical signs indicative of a co-existing vaginal tumor were observed.

Two of the tumors were transplanted after death, utilizing the anterior chamber of the eye as an inoculation site. The technic employed has been described (8).

INCIDENCE

During the 16 year period covered in the present report, approximately 1,100 female rabbits more than 2 years of age came to autopsy and, in each instance, the vagina and cervices were examined. The failure to find cervical growths acquires further significance in view of the fact that the rabbit's uterus is bicornate and contains 2 cervices. Thus, 2,200 cervices were examined without the discovery of a single tumor.

In contrast 3 tumors were found arising at the vaginal squamo-columnar junction. All were epidermoid carcinomas and, in each instance, metastasis had caused the death of the animal. It is of

*This investigation was aided by grants from The Jane Coffin Childs Memorial Fund for Medical Research, The Donner Foundation, and the David, Josephine and Winfield Baird Foundation.

†Fellow of The Jane Coffin Childs Memorial Fund for Medical Research.

††James Hudson Brown Memorial Junior Fellow.

DESCRIPTION OF FIGURES 1 TO 4

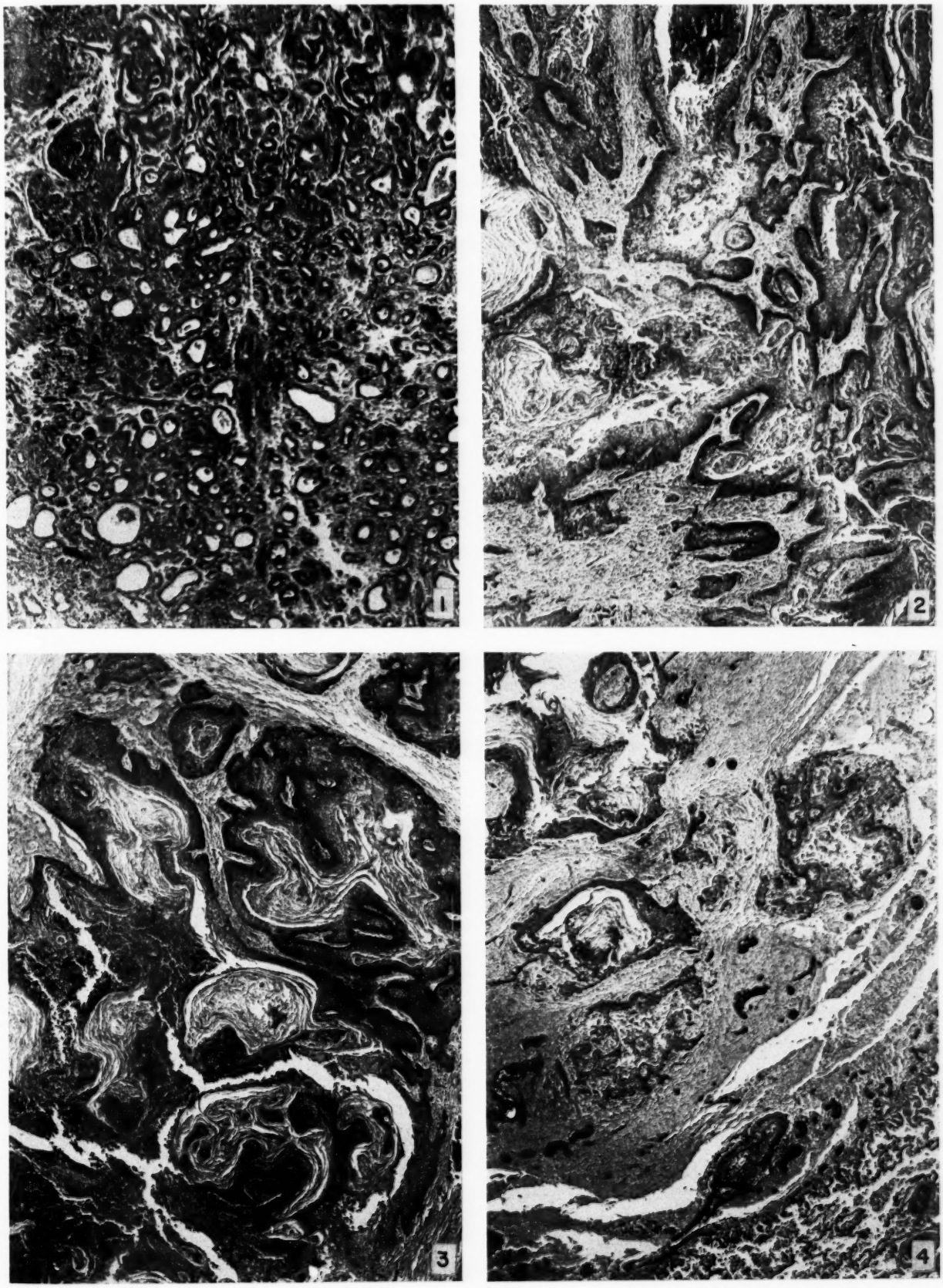
All sections were stained with hematoxylin and eosin.

FIG. 1.—Section of fundic adenocarcinoma in rabbit X773-3. Mag. × 75.

FIG. 2.—Section of epidermoid carcinoma at the squamocolumnar junction in the vaginal wall of rabbit X773-3. Mag. × 35.

FIG. 3.—Section of metastatic epidermoid carcinoma in pleura of rabbit X773-3. Mag. × 35.

FIG. 4.—Section of metastatic epidermoid carcinoma in lung of rabbit X773-3. Mag. × 35.



FIGS. 1-4

interest that 3 extragenital epidermoid carcinomas were discovered in other female rabbits of this same series. One originated in the skin of the cheek (6), another in the skin of the tail and a third in a nipple. Thus, 3 out of 6 epidermoid carcinomas found in the rabbit arose at the vaginal squamo-columnar junction suggesting an increased susceptibility of this region over other bodily parts.

Age.—The ages of the 3 tumor-bearing animals at the time of death were 4 years, 11 months; 5 years, 2 months; and 5 years, 6 months. It should be emphasized that the given ages represent the time of death or of complete autonomous development of the tumors and that nothing is known with reference to the age at inception or the duration of the period of development. Postmortem examination was carried out on 29 females of the same age group during the period of study, indicating an incidence of the tumors in the group of approximately 13 per cent.

Breed.—One of the tumor-bearing animals was a purebred Havana: the others were complex hybrids. The ancestry of one hybrid involved an Havana male that had sired a parent of the purebred doe but the second hybrid bore no genetic relationship. The first hybrid contained Dutch and Polish blood as well, whereas the other was derived from a series of Belgian, Tan and Chinchilla crosses.

Constitution.—All 3 animals had been bred for constitutional study and were known to carry and to transmit genetic abnormalities. The Havana doe represented a concentration in heterozygous form of a variety of hereditary variations under study, the most important being arterio-sclerosis of Monckeberg type, renal aplasia, bile duct adenomas and a uterine anomaly expressed either as a unicornate organ or as an incomplete fusion of horns. The Polish hybrid transmitted cretinism (7) and dwarfism (3); the latter resulting in a diminution of the physique of the animal due to partial expression of the recessive character. The third rabbit belonged to a line known to carry factors concerned in the genesis of mammary cancer. In addition she had been found to transmit splenic reduplication (a condition characterized by the presence of 2 fully formed spleens), a pigmentary deficiency associated with maintenance of downy, infantile fur into adult life and a progressive disorder resulting in premature senility.

Despite such an array of constitutional variations, it should not be assumed that the tumor-bearing animals differed from other rabbits of the colony in this respect, or represented a more abnormal stock than the population of other colonies. In our experience, all rabbits subjected to inbreeding and sufficient study are found to be abnormal in some respect or to transmit some type of physical or functional variation.

The tumor-bearing animals did not share genetic variations of similar type and the role played by constitutional variation in the etiology of the tumors cannot be evaluated from present data.

Fundic adenocarcinoma.—Adenocarcinomas were present in the uterine fundi of all of the animals bearing vaginal tumors. It should be noted, however, that 23 or 79 per cent of the 29 animals in the same age group were similarly affected and the significance of the association may relate to age rather than to some other factor. Widespread endocrinological lesions comparable to those found in rabbits subjected to the long-continued administration of estrogenic substances are present in all animals bearing fundic cancers and it is assumed that the hormonal changes may be of importance in their genesis. Conceivably, the same factors may be operative in the etiology of the vaginal tumors.

Mammary cancer.—Two of the animals showed breast changes comparable with those found in the developmental stages of mammary cancer (2, 4). In one instance the disorder had progressed to the stage of multiple papillomas while in the other, the histological appearance of the tissue sectioned after death of the animal was indistinguishable from cancer. It seems probable that the breast changes in these cases relate to the same hormonal factor concerned in the genesis of the fundic tumors and that their association with vaginal cancers may be on the same basis.

Toxemia of pregnancy.—All 3 animals in this series had recovered from one or more attacks of toxemia of pregnancy. The nature of this disorder in the rabbit has been described (5) and its association with fundic adenocarcinomas noted (1).

DESCRIPTION OF FIGURES 5 TO 10

FIG. 5.—Section at edge of metastatic epidermoid carcinoma in liver of rabbit X773-3. Mag. × 35.

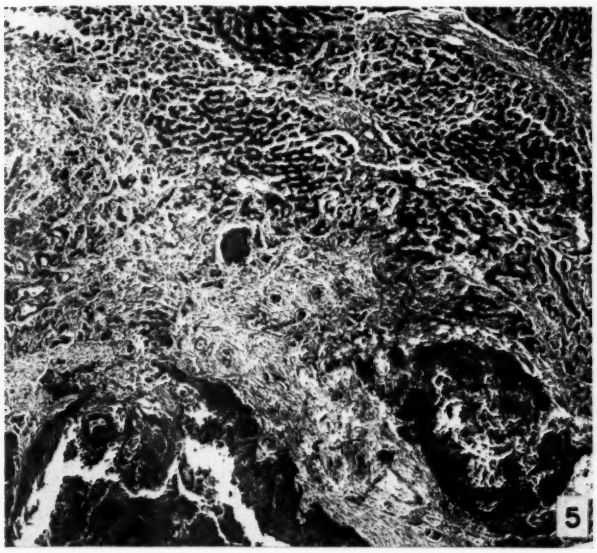
FIG. 6.—Section of fundic adenocarcinoma in rabbit X7634-3. Mag. × 75.

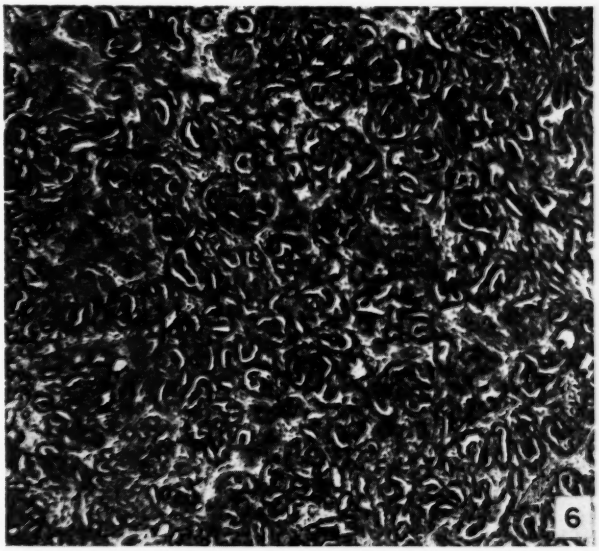
FIG. 7.—Section of vaginal wall at squamocolumnar junction of rabbit X7634-3. Mag. × 14.

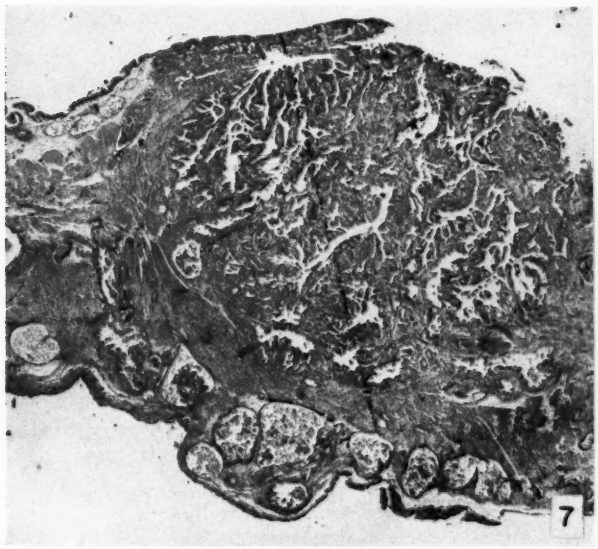
FIG. 8.—Higher power view of epidermoid carcinoma shown in previous photograph. Mag. × 250.

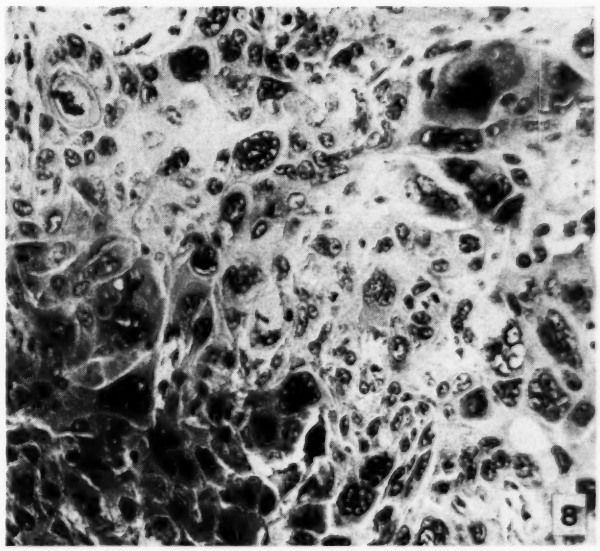
FIG. 9.—Section of metastatic epidermoid carcinoma in diaphragm of rabbit X7634-3. Mag. × 250.

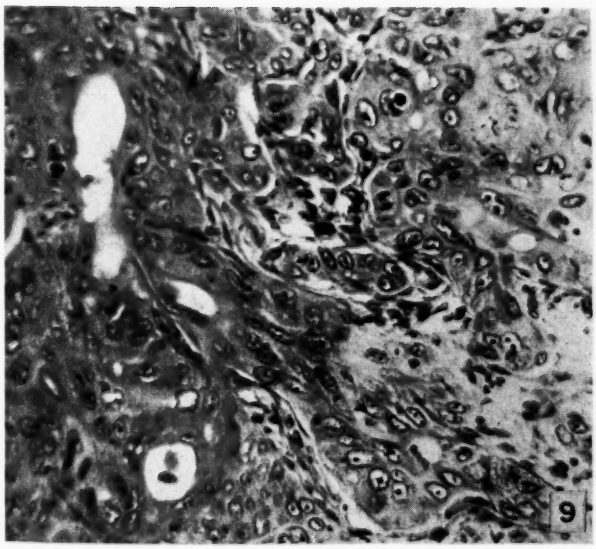
FIG. 10.—Section of metastatic epidermoid carcinoma in mediastinal lymph node of rabbit X7634-3. Mag. × 250.

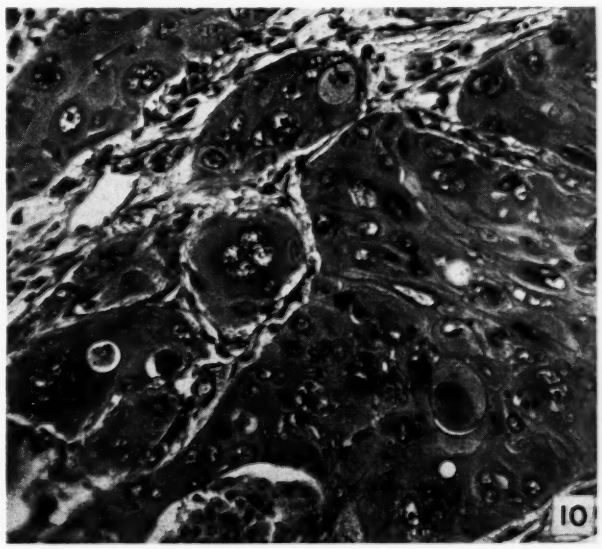












FIGS. 5-10

It would appear to occupy an analogous position in relation to vaginal tumors. The assumption has been made that the liver damage incident to toxemia of pregnancy results in destruction of the cells normally concerned in the inactivation of estrogenic hormone, and that, as a result, the hormone piles up in the circulation to a carcinogenic level.

Clinical History and Pathology

The rabbit, X773-3, a Dutch-Polish hybrid, was first bred at the age of 6 months, and 13 out of the 23 matings carried out during the ensuing 3½ year period proved fertile, resulting in a total of 46 living progeny. The gestation period following the 10th fertile mating was complicated by eclampsia and terminated in a dead-born litter, but no other irregularity distinguished the breeding history of the animal. Approximately a year later, a mass was detected in the uterine fundus and its identity as a developing adenocarcinoma confirmed by laparotomy and histological examination. The animal remained in good condition until a month before death and then, following a rapid weight loss, died at the age of 5 years, 2 months.

At autopsy, confluent masses of soft, necrotic tumor were found arising from the endometrium throughout both uterine horns, and in multiple areas, the growths had invaded and replaced the muscular wall. A firm mass of tumor tissue of different color and consistency was discovered in the posterior vagina opposite the urethral meatus and similar but smaller nodules were scattered irregularly throughout the upper vaginal wall. Metastatic tumor tissue with comparable gross characteristics was present in the omentum, liver, diaphragm, pleura, pericardium and almost completely replaced the lung.

The breeding history of the Belgian hybrid, X7634-3, also began at the age of 6 months and terminated with the discovery of a fundic tumor 3 years later. During this period 7 out of 20 matings were fertile and resulted in 32 living progeny. The course of 2 early pregnancies was associated with acetone breath, urine retention and fetal death but severe eclampsia with convulsions did not occur.

Three laparotomy examinations were made during the 2 year period from discovery of the uterine tumor to death. In each instance, tumor tissue was removed for microscopic study and transfer. Histologically, the tumor presented the usual picture of developing fundic adenocarcinoma. Autologous and homologous transfers were successful and the growth rates corresponded with the developmental stage of the tumor at the time of biopsy.

Several mammary biopsies were performed in this interval to investigate shotty nodules. Histological examination showed cystic disease and papillomas characteristic of the early stages of mammary cancer development in this line.

During the course of physical examination, about the middle of the fifth year of life, the animal's distended bladder was ruptured under pressure of palpation. She was killed immediately and a postmortem examination carried out. The uterus was distended by masses of confluent fungating tumor associated with widespread muscular invasion but without peritoneal extension. A mass of firm, almost cartilaginous tumor encircled the vagina at the squamo-columnar junction and extended upward to the cervix. Small nodules were present in the wall of the urethra and the bladder mucosa was diffusely thickened. In the region of rupture, a mass of tumor protruded into the bladder lumen and extended through the musculature into the peritoneum. Metastatic tumor was found in the inguinal, preaortic and mediastinal lymph nodes, in the diaphragm and in the lung. Other organs were not involved.

The Havana doe, HA550-2, was first bred at the age of 5 months and remained in breeding service for 2¼ years. Seven out of 19 matings proved fertile but 2 of these terminated in fetal resorption and a third in mild toxemia with fetal death. The 4 normal pregnancies resulted in 18 living young.

A laparotomy was performed, toward the middle of the third year of life, to investigate a mass noted in one uterine horn. This proved to be a retained placenta but a pronounced endometrial hyperplasia was found and it is of interest from the viewpoint of pathogenesis that a second laparotomy performed 3 months later disclosed a

DESCRIPTION OF FIGURES 11 TO 16

Fig. 11.—Section of fundic adenocarcinoma in rabbit HA550-2. Mag. × 75.

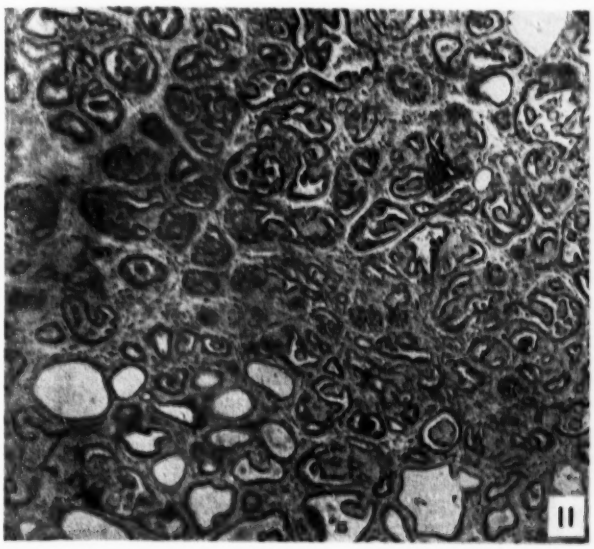
Fig. 12.—Section of anaplastic epidermoid carcinoma at the squamocolumnar junction in the vaginal wall of rabbit HA550-2. Mag. × 250.

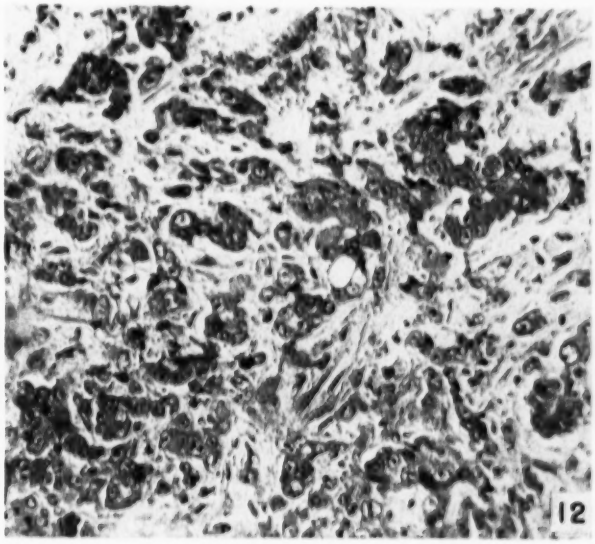
Fig. 13.—Section of aorta embedded in metastatic tumor from rabbit HA550-2. Mag. × 14.

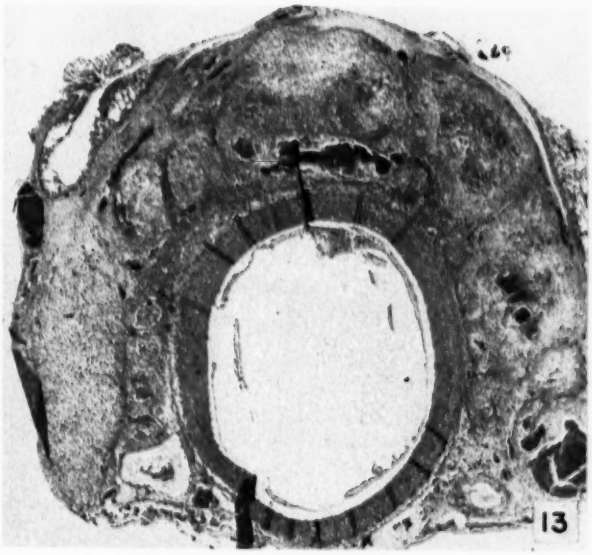
Fig. 14.—Section of metastatic epidermoid carcinoma in inguinal lymph node of rabbit HA550-2. Mag. × 250.

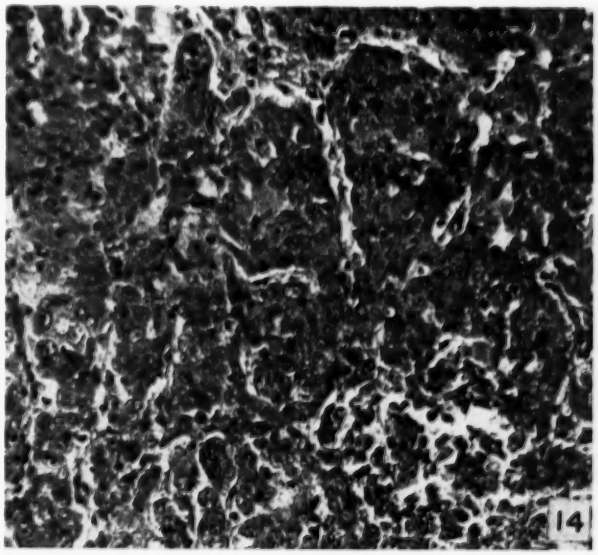
Fig. 15.—Section of metastatic epidermoid carcinoma in lung of rabbit HA550-2. Mag. × 250.

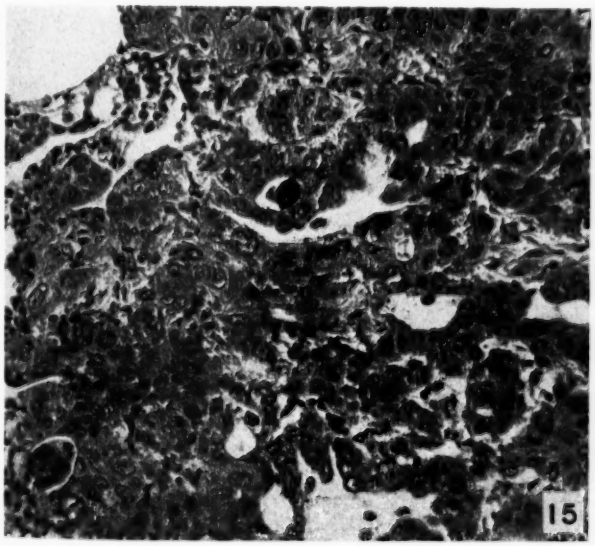
Fig. 16.—Section of metastatic epidermoid carcinoma in femoral bone marrow of rabbit HA550-2. Mag. × 250.

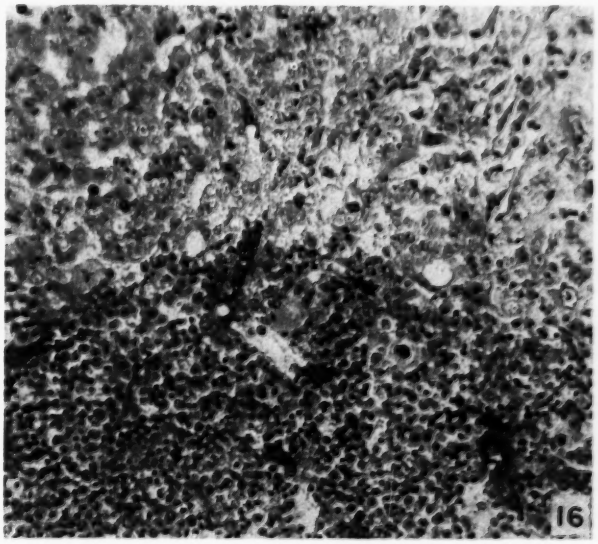












FIGS. 11-16

small endometrial tumor. The development of this tumor was followed throughout the remaining 2 years of life by means of serial biopsies with histological study and transfer of the tissue.

Mammary changes were noted soon after discovery of the uterine tumor. These were also followed by frequent biopsy and the sequence of changes progressing from simple cystic disease through papillomas to morphological cancer was observed. Despite the presence of all of the histological alterations generally considered characteristic of cancer, transfer of the breast tumor to normal animals was never successfully affected.

The rabbit died at the age of 4 years, 11 months, following a rapid weight loss over the period of several weeks. At autopsy, pelvic relations were identified with considerable difficulty. The uterus was replaced by a mass of soft necrotic tumor adherent to the abdominal wall and to the intestine. Innumerable firm nodules were scattered throughout the pelvic peritoneum and mesometrium and, on section, were discovered deep within the fundic tumor. Such nodules were comparable in all respects with the tumor mass found in the vagina. This tumor arose at the level of the urethral meatus, involved the upper two-thirds of the vagina, the urethra and bladder, and extended posteriorly to form a large mass in the pouch of Douglass. Extensions of this mass completely embedded the rectum and the abdominal aorta. The kidneys were hydronephrotic and contained large metastases. Metastases were also present in both ovaries, the spleen, liver, diaphragm, lung, mediastinal nodes, bone marrow and spinal muscles Secondary findings at autopsy were the mammary tumor previously mentioned and a large bile duct adenoma involving the greater part of the left half of the liver.

The histology of the tumors is illustrated in the accompanying figures (Figs. 1 to 17). The tumor found in the rabbit X773-3 was a well differentiated epidermoid carcinoma. The growth in X7634-3 was less differentiated and that in HA550-2 was the most anaplastic of the group. The metastases in all of the animals were epidermoid in type and were derived from the vaginal carcinoma. No metastases from the fundic or mammary tumors were found.

Transplantation of the Vaginal Tumors

Tumor tissue from the animals X7634-3 and HA550-2 was successfully transplanted to the anterior chamber of the eye in normal rabbits. Transfer of the X773-3 tumor was not attempted.

The tissue used for the transfer of the X7634-3 tumor was derived from a mediastinal lymph node. The percentage of takes increased from 50 per cent in the first generation to 80 per cent in the seventh where passage of the tumor was discontinued. The growth rate varied greatly and was not consistently increased with continued transfer. In some instances, the tumor completely filled the anterior chamber of the eye in 27 days while in others the growth had not reached a quarter of this size by the 150th day. Vascularization occurred early in both types of growth, and apparently played no part in the observed variation. Invasion of the iris was also an early occurrence but extension into the vitreous humor did not occur. The histology of the transplants is shown in Figs. 19 and 20.

Transplantation of the mammary tumor from this animal was unsuccessful. Transfer of the fundic cancer resulted in 100 per cent of takes but the growth rate was extremely slow, the tumor consistently filling one half of the chamber in 230 days.

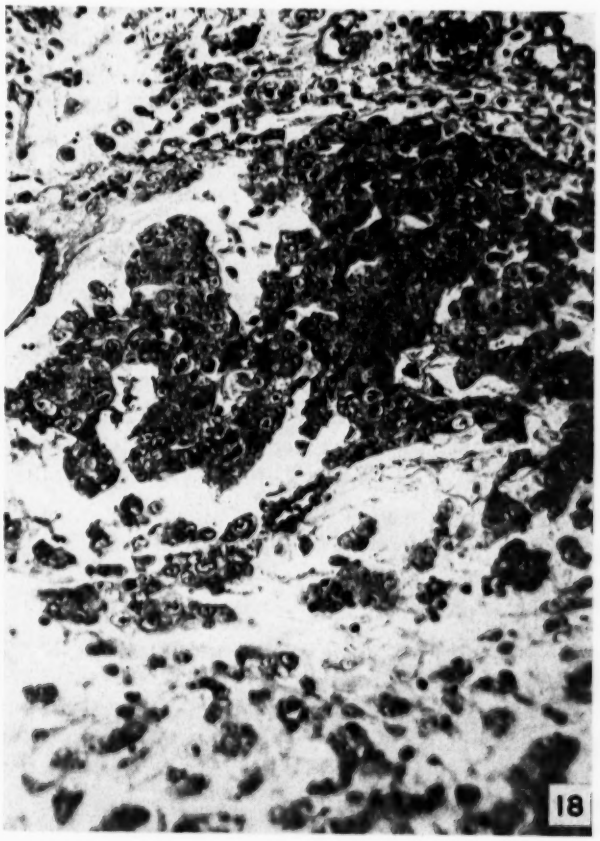
A metastatic liver nodule was utilized in the transfer of the HA550-2 tumor. A hundred per cent of takes was obtained in the first generation and the incidence ranged from 80 to 100 per cent throughout the 14 serial passages of the tumor. The growth rate was rapid and, as a rule, the anterior chamber of the eye was filled by the 20th day. A feature of interest in the transfer of this tumor was the fact that, in many cases, growth occurred without vascularization and continued until the chamber was filled without the appearance of an independent blood supply. Apparently the cells multiplied and grew in the manner of a tissue culture, deriving their nutrient directly from the aqueous humor. Similar observations have been made in the case of other anaplastic cancers and it seems possible that the disorganized appearance of such tumors may be due to a lack of an ability of their cells to stimulate the adequate growth of stroma. The histology of the transplants of this tumor is shown in Fig. 18.

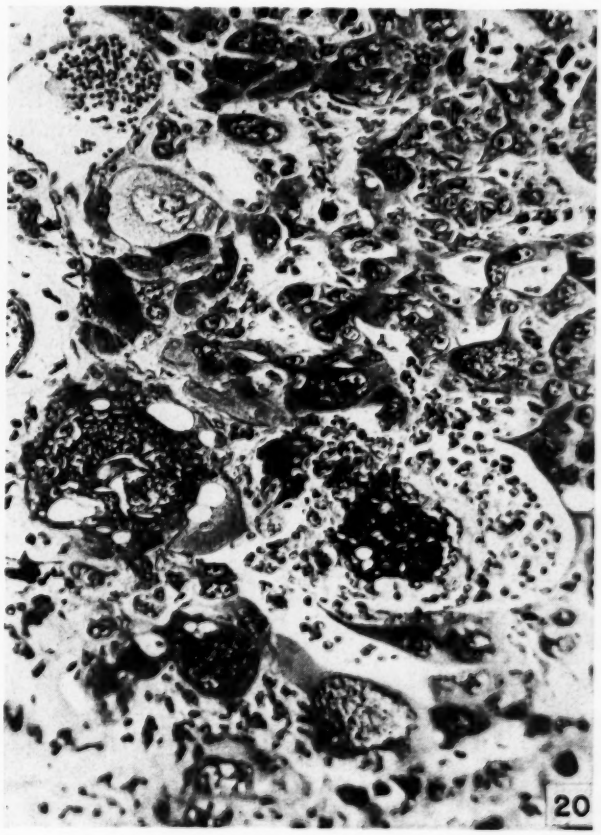
DESCRIPTION OF FIGURES 17 TO 20

Fig. 17.—Section of metastatic epidermoid carcinoma in lung of rabbit HA550-2. Mag. × 250.

Fig. 18.—Section of anterior chamber transplant of epidermoid carcinoma from rabbit HA550-2. Mag. × 250.

Fig. 19 and 20.—Sections of anterior chamber transplants of epidermoid carcinoma from rabbit X7634-3. The bizarre cells seen in Fig. 20 characterized older transplants. Mag. × 250.





FIGS. 17-20

Transfer of the breast tumor of this animal with material obtained at autopsy was unsuccessful. Transfer of the fundic uterine tumor with material taken at autopsy gave rise to 40 per cent of takes in 3 transplanted generations and the growth rate was slow, a period of 160 days being required for the filling of the chamber. The behavior of the fundic tumor at this transfer was in marked contrast to the behavior of tissue obtained from it at a biopsy 4 months before death. At that time, 92 per cent of takes were obtained in 11 transplanted generations and the anterior chambers were consistently filled with tumor by the 45th day. The possible influence of the developing vaginal tumor on the biological behavior of the fundic growth will be considered in detail in a subsequent paper reporting transplantation experiments with a series of multiple tumors in the rabbit.

DISCUSSION

There is little question of the analogy between the squamo-columnar junction on the human cervix and the epithelial junction in the vaginal wall of the rabbit. It is true that both the columnar epithelium of the endocervix and the squamous epithelium of the vagina in man are coelomic in origin, while in all probability, the squamous epithelium of the vaginal wall of the rabbit represents the lining of the urogenital sinus and being derived from ectoderm is embryologically analogous to the external skin. Thus, in man the junction is one between epithelia of common embryological derivation while, in the rabbit, it is actually a meeting of mesothelium and ectodermal epithelium. This difference cannot be considered of great significance in the present connection. The junction in the two species represents the joining of epithelia of different morphological type. Both are subjected to the irritative action of vaginal secretions and the trauma of parturition; neither is stable but fluctuates in location in response to inflammatory stimuli and in effect constitutes a zone of epithelial restlessness.

The occurrence of carcinoma at the squamocolumnar junction in the rabbit despite its different location in this species emphasizes the significance of the junction in the incidence of cervical carcinoma in women.

SUMMARY

Three epidermoid carcinomas arising at the squamocolumnar junction of the vaginal wall of the rabbit have been described. The relatively high frequency of cancer at the squamocolumnar junction in both man and the rabbit despite its different location in the two species emphasizes the significance of the junction as a predisposing factor in carcinogenesis.

REFERENCES

1. Greene, H. S. N. Uterine Adenomata in the Rabbit III. Susceptibility as a Function of Constitutional Factors. J. Exper. Med., **73**:273-292. 1941.
2. Greene, H. S. N. Familial Mammary Tumors in the Rabbit. I. Clinical History. J. Exper. Med., **70**: 147-158. 1939.
3. Greene, H. S. N. A Dwarf Mutation in the Rabbit. The Constitutional Influence on Homozygous and Heterozygous Individuals. J. Exper. Med., **71**: 839-856. 1940.
4. Greene, H. S. N. Familial Mammary Tumors in the Rabbit. II. Gross and Microscopic Pathology. J. Exper. Med., **70**:159-166. 1939.
5. Greene, H. S. N. Toxemia of Pregnancy in the Rabbit. I. Clinical Manifestations and Pathology. J. Exper. Med., **65**:809-832. 1937.
6. Greene, H. S. N., and Brown, W. H. A Transplantable Squamous Cell Carcinoma in the Rabbit. Cancer Research, **3**:53-64. 1943.
7. Greene, H. S. N., Hu, C. K., and Brown, W. H. A Lethal Mutation in the Rabbit with Stigmata of an Acromegalic Disorder. Science, **81**:25-26. 1935.
8. Greene, H. S. N., and Saxton, J. A., Jr. Uterine Adenomata in the Rabbit. I. Clinical History, Pathology and Preliminary Transplantation Experiments. J. Exper. Med., **67**:691-708. 1938.

Strain Differences in Response to Diethylstilbestrol and the Induction of Mammary Gland and Bladder Cancer in the Rat

W. F. Dunning, Ph.D., M. R. Curtis, Ph.D., and A. Segaloff, M.D.*

(Department of Pathology, Wayne University College of Medicine in cooperation with the Detroit Institute of Cancer Research, Detroit 26, Michigan)

(Received for publication February 17, 1947)

For the past 20 years, the field of experimental cancer research has been largely dominated by studies on the occurrence, production, or prevention of mammary cancer in the mouse. The development through inbreeding and selection of numerous strains of mice in which these neoplasms occur spontaneously with a relative high frequency and the economy of feeding and housing mice in preference to other laboratory animals have been factors contributing to this situation. So predominate is this particular form of malignant disease that mice that fail to develop it have become known as non-tumor mice, irrespective of any further tumor history or the ease with which they respond to various tumor-inducing agents. The controversy over the hereditary nature of these tumors was largely explained by the discovery of Little and his co-workers (8) and of Korteweg (12) of an important extrachromosomal or milk influence. This was demonstrated by significant differences in the incidence of mammary tumors in the F_1 progeny of reciprocal crosses between high and low mammary cancer stocks. Bittner (2, 3) demonstrated further, that foster nursing of the progeny of cancerous mothers by low mammary cancer stock mothers largely prevented the occurrence of mammary cancer in these foster-nursed females. More recently Bittner (4) has shown that feeding the filtrate from macerated lactating mammary glands obtained from mice of high mammary cancer stock caused the foster-nursed daughters to develop mammary cancer 8 to 10 months later. Green, Moosey and Bittner (9) have further demonstrated the production of antibodies and antiserums, which are capable of neutralizing or inactivating the centrifugates of mouse mammary cancer, while antiserums produced similarly in response to normal mouse tissue are ineffective. Thus an important factor in mouse mammary cancer development appears to be a self-perpetuating, exogenous body analogous to a virus.

Early experiments in castration demonstrated the relationship between the ovarian hormones and the development of mammary cancer in the mouse. Prepubertal ovariectomy inhibited the development of mammary tissue and prevented the occurrence of mammary cancer while the injection of estrogens caused the mammary cancers to appear earlier than they did in untreated females. Lacassagne (10, 11) showed that the injection of estrogens in male mice from birth or an early age caused them to develop mammary cancer in the same frequency as their sisters. These findings have been confirmed by many investigators as indicated in a recent survey of the literature on the subject by Gardner (7). It seems however, that the female sex hormones alone are insufficient to cause the development of mammary cancer in some mice that lack the hereditary factors. Thus the development of mammary cancer in the mouse appears to be dependent upon three separate etiological factors; namely, hereditary, extrachromosomal or milk, and hormonal. This anomalous situation has given impetus to a search for similar factors in the etiology of other types of neoplasia in the mouse and of mammary cancer in other species, including man. So far, the search has failed to identify in other species a factor analogous to the milk influence in mammary cancer of mice.

The rat has been considered peculiarly insusceptible to mammary cancer but, although Curtis, Bullock and Dunning (5) reported only 2 mammary cancers in a colony of nearly 9,000 female rats of tumor age, the animal has not proved resistant to the induction of mammary cancer by excessive estrogenic stimulation. The contrast between the relatively low frequencies of induced mammary cancer reported by McEuen (13) and Eisen (6) and the high frequency obtained by Geschickter and Byrnes (8) and Nelson (14) would suggest the operation of

*Present address: The Alton Ochsner Medical Foundation, New Orleans 15, La.

possible hereditary factors, since these investigators employed different strains of rats. McEuen reported only 2 mammary cancers in 12 rats that survived for 500 days with daily injections of 30 γ of estrone and Eisen obtained 2 mammary cancers in 103 rats that survived for 242 days with implantations of 1 to 20 mgm. of crystalline estradiol dipropionate in paraffin. In contrast, Nelson observed 63 mammary cancers in 103 treated rats that survived 300 days and longer. Geschickter and Byrnes reported 202 rats with mammary cancer from 555 rats treated with various doses of estrone and stilbestrol and prescribed that, to produce mammary cancer in the rat, the dose of estrogen must be well beyond the physiologic limit (10 or more times the threshold dose) and the treatment continuously applied for a period of months (30 or more times the duration of normal estrus). Since the dose of estrogen employed by McEuen and by Eisen met these requirements, there would seem to be some fundamental difference in the rats used for these investigations. Obviously, unphysiological estrogenic stimulation is a factor in the etiology of mammary cancer in the rat as well as in the mouse. Here would seem to be an excellent opportunity to determine further, whether or not hereditary or milk factors predispose rats to estrogen-induced mammary cancer.

It is the purpose of the present paper to report the response of 3 inbred lines of rats to unphysiological stimulation from diethylstilbestrol. A later communication will deal with the response of reciprocal F_1 hybrids between 2 of these lines to an effective mammary cancer-inducing dose of diethylstilbestrol.

MATERIALS AND METHODS

Pedigreed rats of 3 distinct lines, Fischer line 344, Copenhagen line 2331, and A × C line 9935, were used for this investigation. They received the laboratory stock diet (Friskie Dog Pellets) supplemented with a green vegetable once a week. The cancer history of the ancestors of these three lines is known for 40, 31 and 27 brother-by-sister generations, respectively, and no spontaneous mammary cancers were observed. Each series consisted of 30 males and 30 females from each line, which at the start of the experiment were between 3 and 4 months of age.

In Series I, pellets weighing between 15 and 25 mgm. of compressed crystalline diethylstilbestrol[1] were implanted in the scapular region by means of nasal forceps inserted through an incision in the skin of the lower back. The incision was closed with a wire staple. The rats were weighed and inspected for mammary tumors every 2 weeks. Any rat that gained appreciably for 2 successive weighings, or any male in which the testicles descended, or any female found to be in a diestrous phase, was reimplanted with another pellet of diethylstilbestrol. At death any remaining pellet was removed, weighed and subtracted from the weight of diethylstilbestrol that had been administered to the rat. At the postmortem examination, a thorough inspection for gross tumors was made and gross sections of mammary gland, thymus, liver, kidney, adrenals, urinary bladder, sex glands, and pituitary were preserved for microscopic examination.

In Series II, a similar group of rats were implanted in the scapular region with pellets composed of 75 per cent cholesterol and from 4 to 15 mgm. of diethylstilbestrol. The diethylstilbestrol in this series was much more slowly absorbed and no reimplantations were necessary to keep the rat in a constant state of hyperestrinism for the remainder of its life. It was, however, impossible to determine the amount of diethylstilbestrol actually absorbed from the pellets because at the death of the animal, after many months in the rat, some of the pellets weighed the same or more than when they were implanted. Otherwise, these rats were treated similarly to those of Series I.

RESULTS

The results are summarized briefly in Tables I and II and shown graphically in Figs. 1 and 2. The rats of Series I represented in Table I and Fig. 1 showed considerable variation in survival and in the absorption of diethylstilbestrol. The 17 Fischer line 344 females on which complete records were obtained survived for an average of only 67 days and had absorbed 19 mgm. of crystalline diethylstilbestrol or an average of 0.26 mgm. per rat per day. The average body weight fell from 120 gm. to 110 gm. in the first 2 weeks and remained low. One female survived for 143 days, having absorbed 34 mgm. of diethylstilbestrol. All of these females died with pyometra and in most cases peritonitis was evident. The Fischer line 344 males lived twice as long, or an average of 136 days, absorbing an average of 29 mgm. of diethylstilbestrol at the rate of 0.2 mgm. per rat per day. Their average body weight fell from 167 to 144 gm. within 2 weeks and only 4 of the rats survived for 6 months. The Copenhagen line 2331 and A × C line 9935 rats absorbed the diethyl-

[1]Supplied through the courtesy of Dr. D. F. Robertson of Merck and Co., Rahway, N. J.

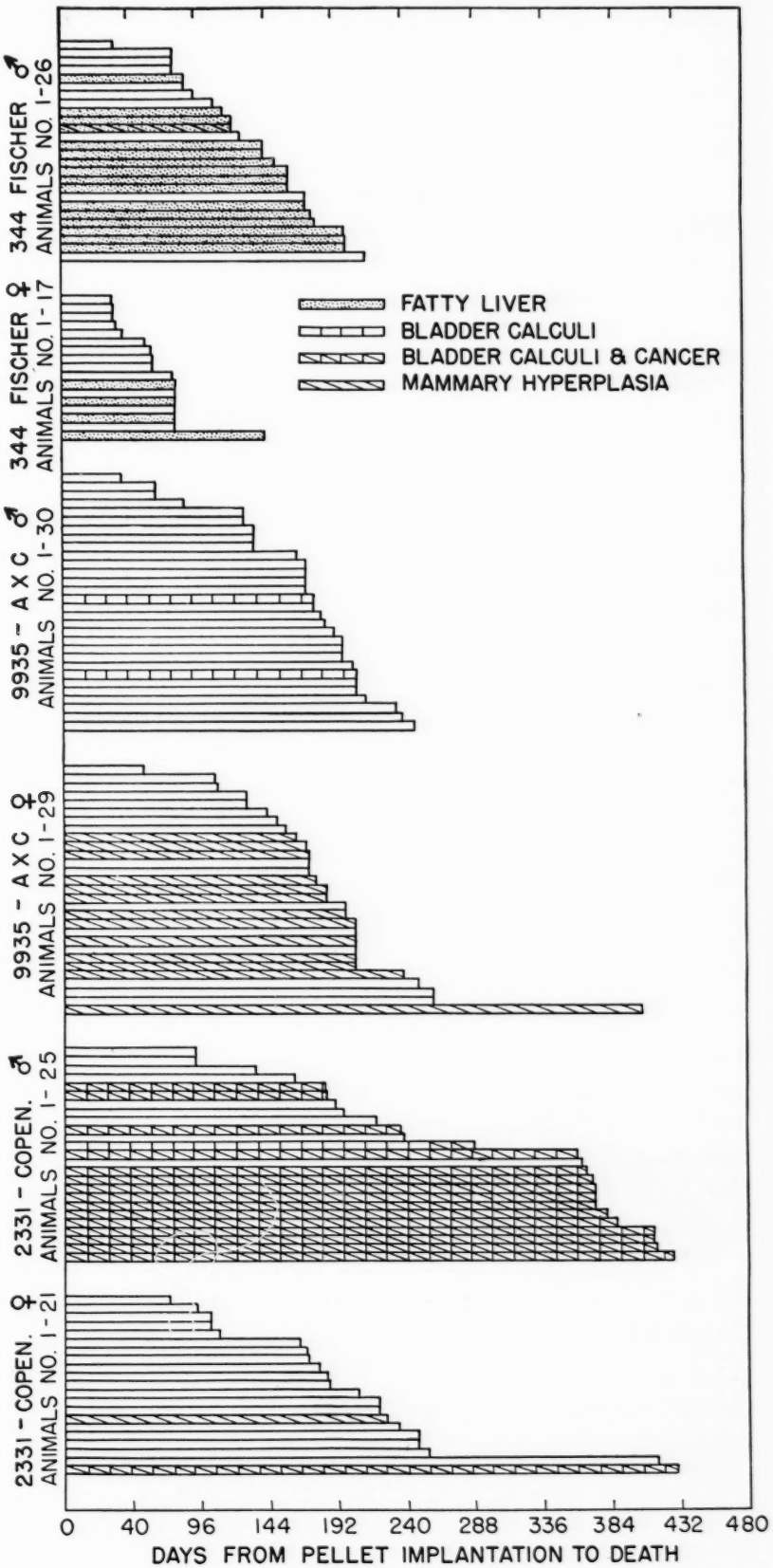

344 FISCHER ♂ ANIMALS NO. 1-26
344 FISCHER ♀ ANIMALS NO. 1-17
9935 - A X C ♂ ANIMALS NO. 1-30
9935 - A X C ♀ ANIMALS NO. 1-29
2331 - COPEN. ♂ ANIMALS NO. 1-25
2331 - COPEN. ♀ ANIMALS NO. 1-21
FATTY LIVER
BLADDER CALCULI
BLADDER CALCULI & CANCER
MAMMARY HYPERPLASIA
0 40 96 144 192 240 288 336 384 432 480
DAYS FROM PELLET IMPLANTATION TO DEATH

FIG. 1.—Survival period and tumor history of rats of each strain with pellets of crystalline diethylstilbestrol implanted in subcutaneous tissues of scapular region. (Each rat is represented by a bar, length of which shows the period of survival in days, with postmortem findings indicated by proper shading.)

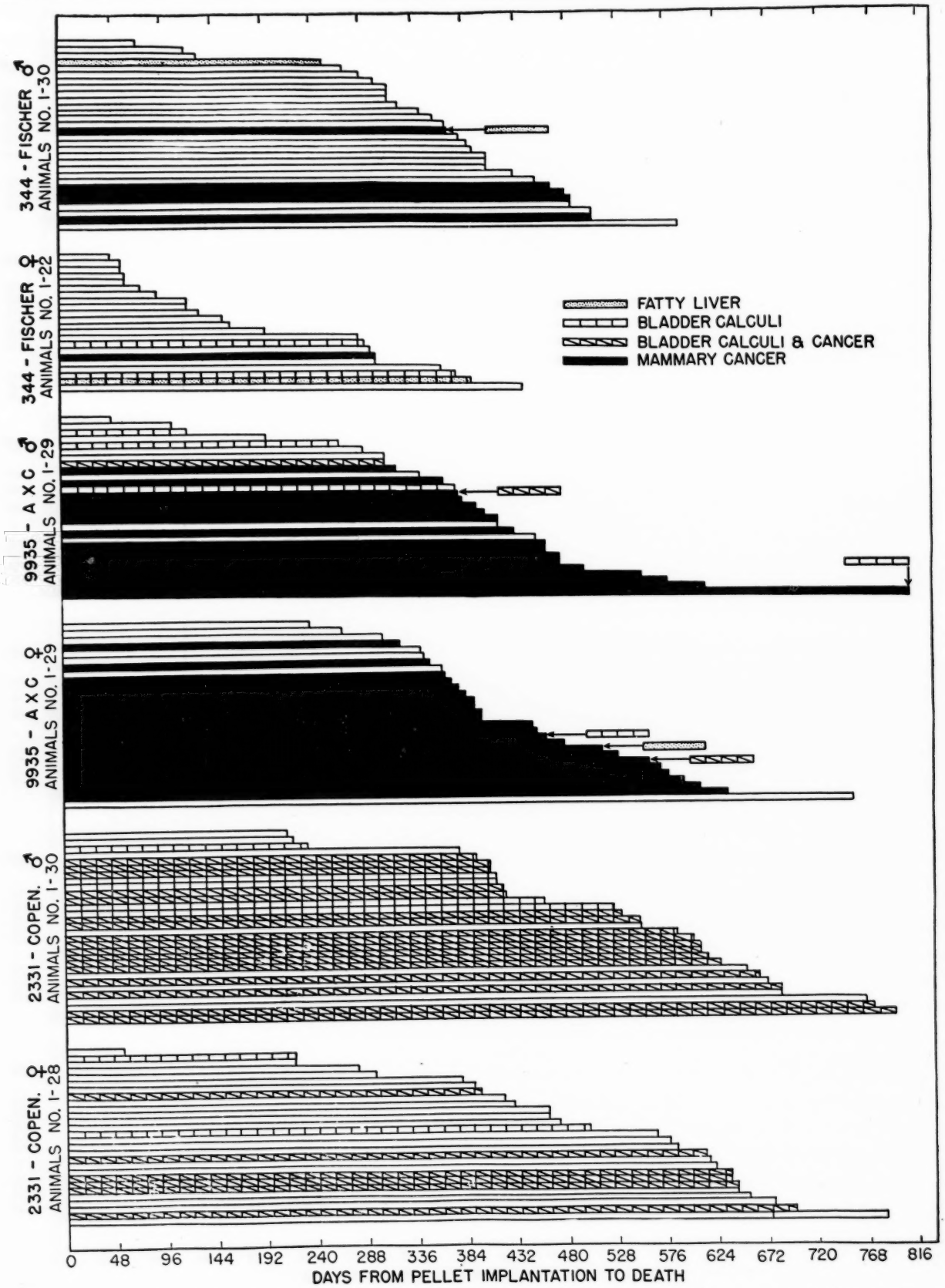

344 - FISCHER ♂
ANIMALS NO. 1-30
344 - FISCHER ♀
ANIMALS NO. 1-22
9935 - A X C ♂
ANIMALS NO. 1-29
9935 - A X C ♀
ANIMALS NO. 1-29
2331 - COPEN. ♂
ANIMALS NO. 1-30
2331 - COPEN. ♀
ANIMALS NO. 1-28
FATTY LIVER
BLADDER CALCULI
BLADDER CALCULI & CANCER
MAMMARY CANCER
0 48 96 144 192 240 288 336 384 432 480 528 576 624 672 720 768 816
DAYS FROM PELLET IMPLANTATION TO DEATH

FIG. 2.—Survival period and tumor history of rats of each strain with cholesterol pellets containing 25 per cent diethylstilbestrol implanted in subcutaneous tissues of the scapular region. (Each rat is represented by a bar, length of which shows the period of survival in days, with post-mortem findings indicated by proper shading. In the cases where rat had mammary cancer and another of the pathological conditions, the latter is indicated by a box with proper shading connected by an arrow.)

stilbestrol more slowly and survived the treatment longer. There was less difference between the A × C males and females, but the female had a longer average survival because one female lived for 406 days, as shown in Fig. 1. The average survival period was 167 days for the males and 186 days for the females, while the former absorbed 24 mgm. of diethylstilbestrol and the latter an average of 25 mgm. The average body weight dropped from 142 gm. to 117 gm. in the males and in the females from 116 gm. to 104 gm. within 2 weeks after the pellets were implanted. The Copenhagen line 2331 males survived the treatment longer than any of the other rats—an average of 289 days compared with 200 days for the females. The former absorbed 38 mgm. of diethylstilbestrol and the latter 31 mgm. The average body weight of the males fell from 122 gm. to 94 gm. in the first 2 weeks and that of the females from 115 gm. to 94 gm. Eleven males and 2 females survived for more than a year.

No mammary cancers were observed in these rats, although considerable hyperplasia of the mammary tissue was noted in 13 A × C females, also in one Fischer male and 2 Copenhagen females. The most conspicuous differences were observed in lesions of the pituitary, liver and bladder. The Fischer line 344 males and females without exception had large hemorrhagic pituitary adenomas, which in some cases weighed as much as 500 mgm. The Copenhagen line 2331 rats had enlarged pituitaries, which rarely became hemorrhagic and averaged less than 100 mgm., although they lived significantly longer than the Fischers and absorbed more diethylstilbestrol. The pituitaries in the A × C line 9935 rats were intermediate, rarely obtaining the size of those in the Fischers and usually exceeding those of the Copenhagens. A previous publication (16) noted a similar difference in response of the pituitary and also a distinct difference in the adrenals of A × C and Fischer castrated males to daily injections of 1 mgm. of diethylstilbestrol. The characteristic difference between the pituitary adenomas in the Fischer line 344 and the Copenhagen line 2331 rat is shown in Fig. 3. The albino rat on the right, a Fischer line 344 male, had absorbed 33 mgm. of diethylstilbestrol in 170 days. The pituitary in this rat weighed 400 mgm. The rat on the left was a Copenhagen female which had absorbed 34 mgm. of diethylstilbestrol in 183 days. The pituitary weighed 60 mgm. The photograph also shows the pellets *in situ*.

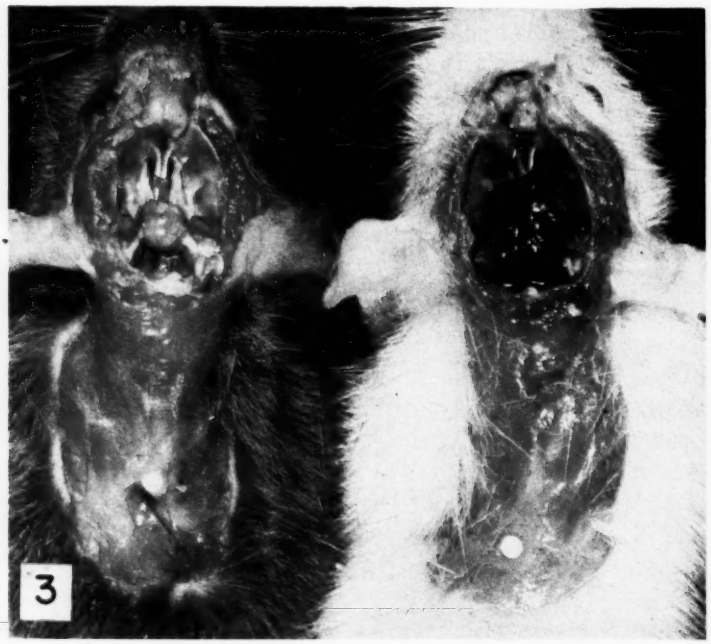
One of these large hemorrhagic pituitary adenomas from a Fischer line 344 male was transplanted into the subcutaneous tissues of 6 males of the same line in which diethylstilbestrol pellets had been previously implanted. All of the grafts grew progressively until the hosts died from the effects of the rapid absorption of diethylstilbestrol with large pituitaries *in situ*. Some of the subcutaneous growths attained a diameter of 1.5 cm. and weighed nearly 1 gm. A section through one of these subcutaneous growths is shown in Fig. 9. It has the characteristic large sinuses filled with red blood cells, is quite cellular with many mitoses and in many respects resembles a neoplasm, but it failed to grow upon transplantation into untreated rats of the same inbred line.

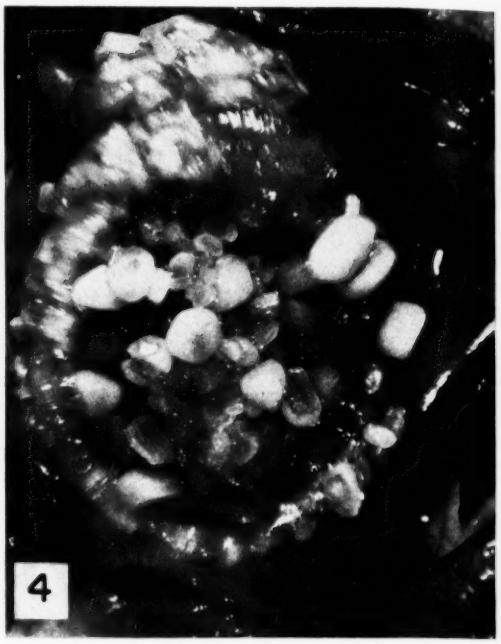
Extensive fatty infiltration of the liver was observed in 16 of the Fischer line 344 males and in 4 of the females. Fatty livers were not found in treated rats of the other 2 lines nor in untreated Fischer rats. A section through one of these livers is shown in Fig. 7.

The most striking lesion was observed in the bladder of the Copenhagen line 2331 male rats which had been treated for 6 months and more. The bladder was distended up to 10 times its normal size, and in many instances was packed with faceted calculi. These, usually smooth and rounded, but sometimes rough and crystalline, proved to be chiefly magnesium ammonium phosphate like those reported by Benjamin, Wilson and Leahy (1) for α-estradiol-treated rats. The walls of the bladder were usually thickened and papillary and in 14 of the 16 males with calculi, contained one or multiple papillomas. One female Copenhagen had bladder calculi and papilloma and two A × C males also had bladder calculi.

TABLE I: NUMBER OF RATS OF EACH STRAIN, AVERAGE DOSE OF DIETHYLSTILBESTROL, AVERAGE SURVIVAL, AND PITUITARY WEIGHT, AND NUMBER OF RATS WITH OTHER PATHOLOGICAL LESIONS

No. of rats	Sex	Strain	Av. no. days until death	Av. amt. of stilbestrol (*mgm.*)	Av. daily absorption of stilbestrol (*mgm.*)	Average pituitary wt. (*mgm.*)	Pathological Lesions		
							Fatty liver	Mammary gland hyperplasia	Bladder calculi
26	♂	Fischer	136	28.6	0.20	405	16	1	0
17	♀	Fischer	67	18.8	0.26	309	4	0	0
30	♂	A × C	167	24.1	0.14	165	0	0	2
29	♀	A × C	186	25.4	0.14	161	0	13	0
25	♂	Copenhagen	289	38.3	0.13	98	0	0	16
21	♀	Copenhagen	200	30.5	0.15	61	0	2	1





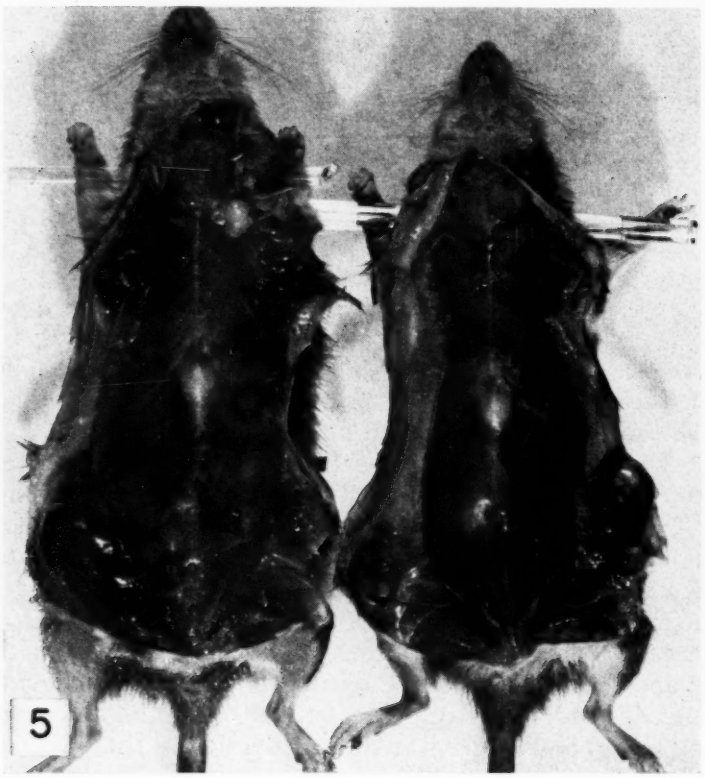
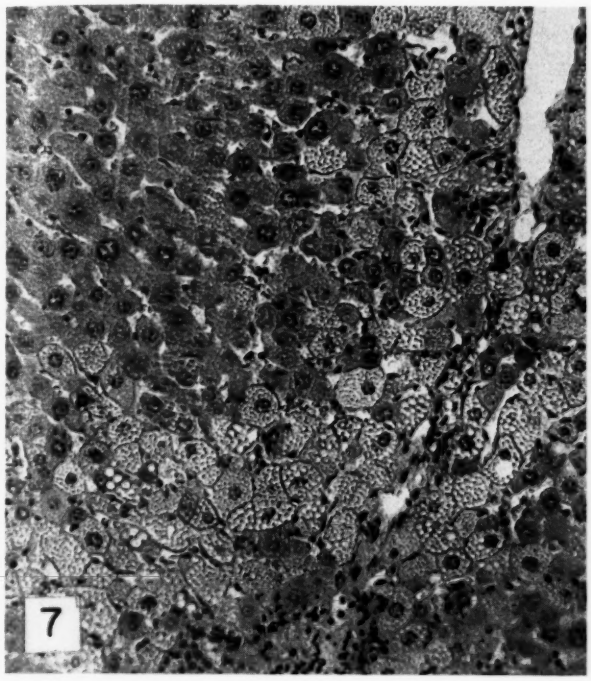


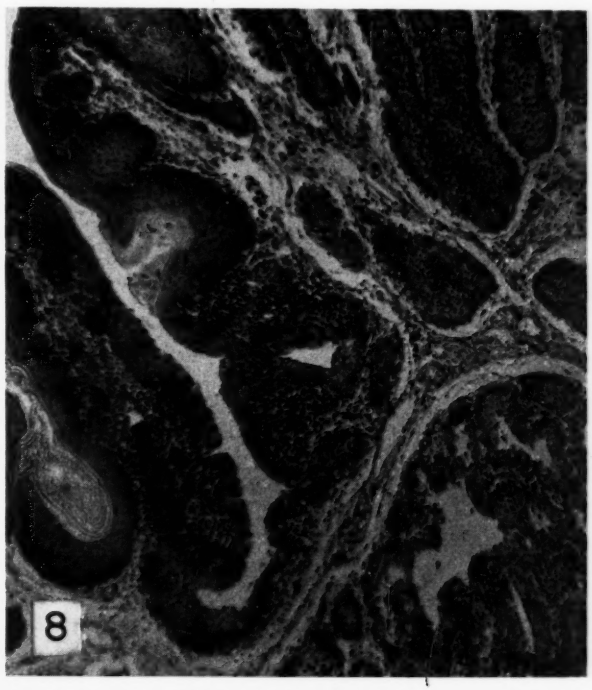
FIG. 3.—Pituitary adenoma and diethylstilbestrol pellets *in situ*. *White rat* = Fischer line 344 male which absorbed 33 mgm. of crystalline diethylstilbestrol in 170 days. *Agouti rat* = Copenhagen line 2331 female which absorbed 34 mgm. of crystalline diethylstilbestrol in 183 days.

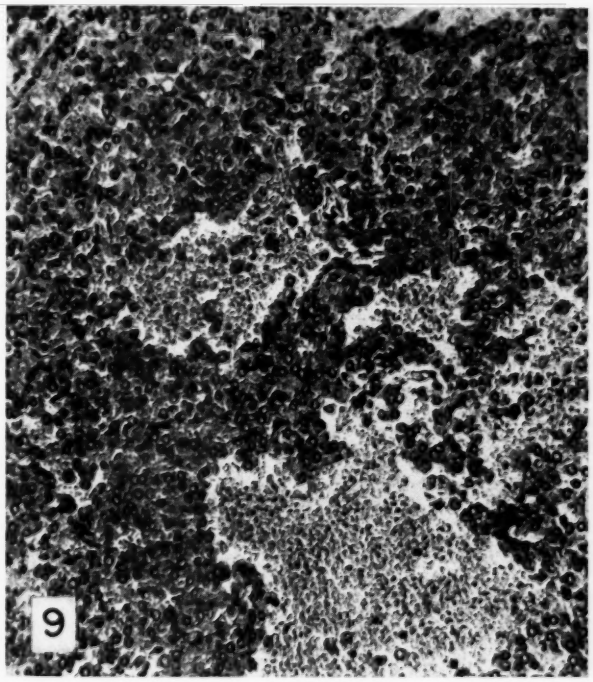
FIG. 4.—Calculi exposed in the bladder of an A× C line 9935 rat 308 days after receiving a cholesterol pellet containing 12 mgm. of diethylstilbestrol.

FIG. 5.—A × C line 9935 rats with induced mammary cancers. At right, female after 371 days with 5 mgm. of diethylstilbestrol in cholesterol pellet. At left, female after 377 days with 7.5 mgm. of diethylstilbestrol in cholesterol pellet.

FIG. 6.—A × C line 9935 female with induced lymphosarcoma 322 days after receiving 4 mgm. of diethylstilbestrol in cholesterol pellet.







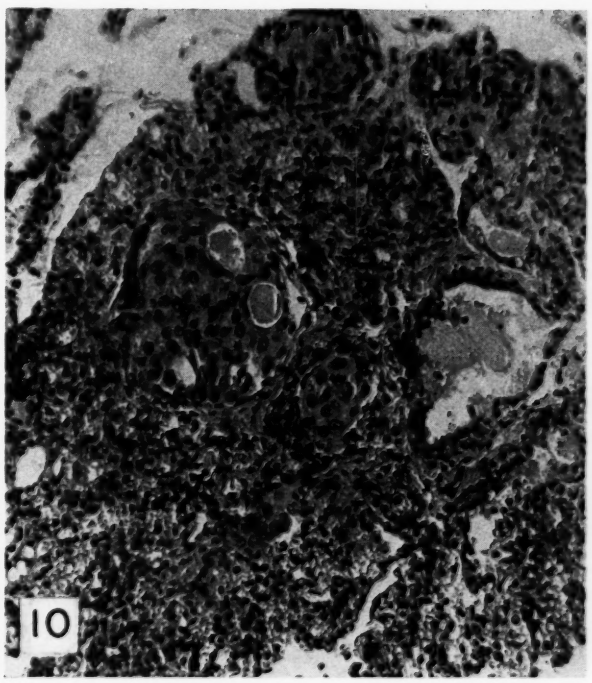


FIG. 7.—Section of a fatty liver from a Fischer line 344 rat which absorbed 33 mgm. of diethylstilbestrol in 177 days. Mag. × 125.

FIG. 8.—Section of a bladder papilloma in a A × C rat 308 days after it received 12 mgm. of diethylstilbestrol in a cholesterol pellet. Mag. × 50.

FIG. 9.—Section of a subcutaneous transplant of a pituitary adenoma from a Fischer line 344 rat, 198 days after transplantation. Mag. × 125.

FIG. 10.—Precancerous lesion from the mammary gland of an A × C male rat, 308 days after it received 14.0 mgm. of diethylstilbestrol in a cholesterol pellet. Mag. × 150.

Fig. 4 shows one of these bladders with the calculi exposed and Fig. 8 shows a section through one of the papillomas. Unlike the urinary calculi reported by Wilson, Benjamin and Leahy (19) in rats, and Schenken, Burns and McCord (15) in α-estradiol-treated C3H mice, these did not appear to be associated with inflammatory changes in the urinary tract. No calculi were found in the kidneys

or ureters and pathological changes were no more frequent in the kidneys of these rats than in those of the treated rats of the 2 other lines.

The rats of Series II, which received the pellets composed of 25 per cent diethylstilbestrol and 75 per cent cholesterol tolerated the treatment much longer as shown in Table II and Fig. 2. Half of the Fischer line 344 males and 4 of the females survived for a year. The majority of the rats of the two other lines lived more than a year and 2 A × C and 4 Copenhagen rats survived for more than 2 years. The Fischer and A × C females lost an average of only 2 to 4 gm. during the first 2 weeks after the pellets were implanted whereas the Copenhagen females lost 10 gm. The males lost from 16 to 20 gm. during the interval, but their weight gradually increased after the first 4 months. Very few fatty livers were observed, 2 in Fischer line 344 males, 1 in a Fischer and 1 in A × C rats. The difference between the Fischer line 344 and A × C line 9935 rats is partly attributable, as shown in Fig. 2, to the differences in survival, but not entirely. The tumors observed in the Fischer line 344 rats were all solitary as shown in Table II, but in the A × C rats most of the tumors were multiple. Typical examples are shown in Fig. 5. The rat at the left of the photograph had 6 probably independent tumors and the one on the right had 3. Fourteen independent tumors were found in one female; and many of the rats had additional areas of extreme hyperplasia with ductal infoldings and squamous metaplasia as shown in Fig. 10, which were considered precancerous lesions. In the 22 A × C line 9935 female tumor-bearers, 89 tumors were observed or an average of 4 to the rat. In the 17 A × C males 39 tumors were described or an average of 2 to the rat. One A × C male had 5 gross tumors and one

TABLE II: NUMBER OF RATS OF EACH STRAIN, AVERAGE DOSE OF DIETHYLSTILBESTROL IN CHOLESTEROL, AVERAGE SURVIVAL, AND NUMBER OF RATS WITH MAMMARY GLAND CANCER AND BLADDER CALCULI AND CANCER

No. of rats	Sex	Strain	Av. no. days until death	Av. amt. of stilbestrol (*mgm.*)	Average pituitary wt. (*mgm.*)	Mammary gland		Bladder	
						No. of rats	No. of cancers	No. with calculi	No. with cancer
30	♂	Fischer	367	7.7	147	5	5	0	0
22	♀	Fischer	200	7.5	104	1	1	3	0
29	♂	A × C	388	7.4	155	17	39	6	2
29	♀	A × C	442	5.4	116	22	89	1	1
30	♂	Copenhagen	528	8.6	64	0	0	22	16
28	♀	Copenhagen	499	9.0	92	0	0	8	6

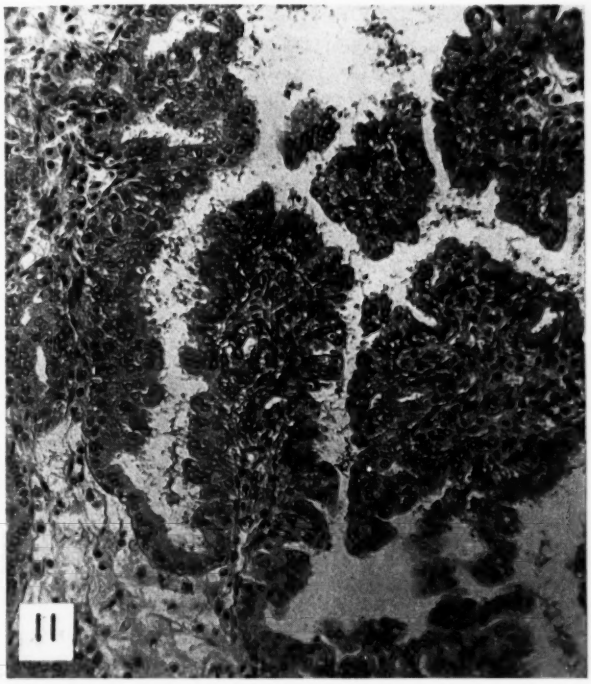
an A × C female. The pituitaries were enlarged but not as consistently or as rapidly, or to as great a magnitude as in the rats of the other series.

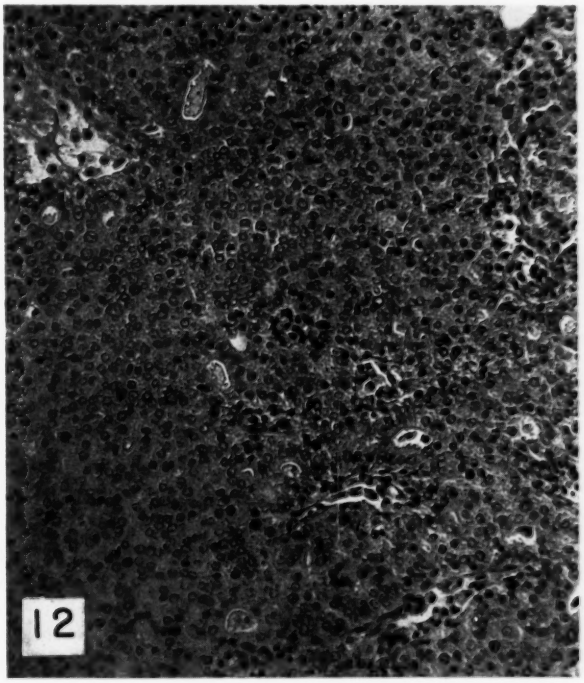
Bladder calculi were observed in 22 or 73 per cent of the 30 Copenhagen line 2331 males and in 8 or 30 per cent of the 28 females of this line. The first were observed in a male that died 231 days after the pellet was implanted. In Series I, the calculi were found consistently in the Copenhagen line 2331 males after 180 days. Six A × C line 9935 males and 1 female also had calculi and a few small crystals were observed in the bladder of 3 of the Fischer line 344 females. Bladder papillomas and grade I squamous cell cancer were observed in 16 of the Copenhagen line 2331 males and 6 of the females which had calculi. Two A × C line 9935 males and 1 female also had bladder papillomas and cancer.

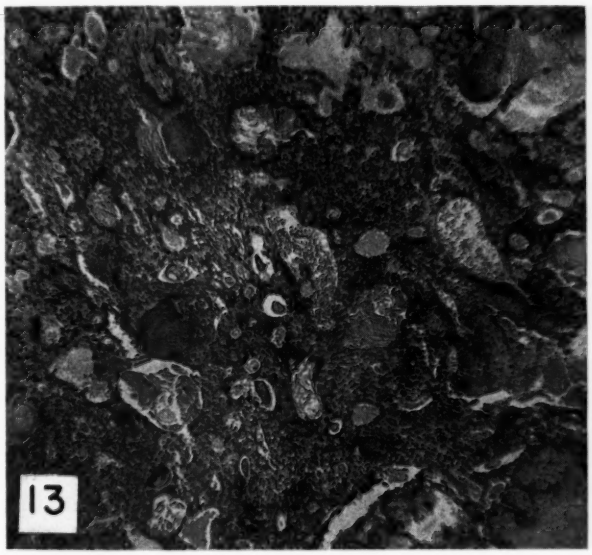
In Series II, mammary cancer occurred in 22 or 85 per cent of the females and in 17 or 80 per cent of the males of A × C line 9935. One Fischer line 344 female and 5 Fischer males also had mammary cancers. No mammary cancers were found in the treated Copenhagen males or females, although they outlived the Fischer line 344 rats by several months and lived fully as long as the other which was identified by microscopic examination of the mammary tissue. Fig. 6 shows an A × C female rat with a lymphosarcoma which arose in the superficial lymph nodes of the left groin breast and metastasized to the other superficial lymph nodes, mediastinum and lungs. Fig. 14 shows a section through this tumor. The mammary cancers[2] in the A × C rats included 107 papillary cystic adenocarcinomas (Fig. 11), 11 adenocarcinomas and squamous cell cancers or adenocanthomas (Fig. 13), 3 solid carcinomas (Fig. 12), 2 solid carcinomas with papillary areas and 4 that were unclassified. The mammary tumors in the Fischer line 344 rats included 1 solid carcinoma, 1 interductal carcinoma and 4 papillary cystic adenocarcinomas.

No pellet was found in the A × C female shown in Fig. 2, which outlived the others and died without a mammary cancer. Scantily developed mammary tissue and a small pituitary (10 mgm.) suggested that the pellet had ulcerated out a considerable time before. Postmortem examination revealed an extensive osteosarcoma of the uterus

[2]The authors gratefully acknowledge indebtedness to Dr. Martha E. Madsen for assistance in classifying the tumors.







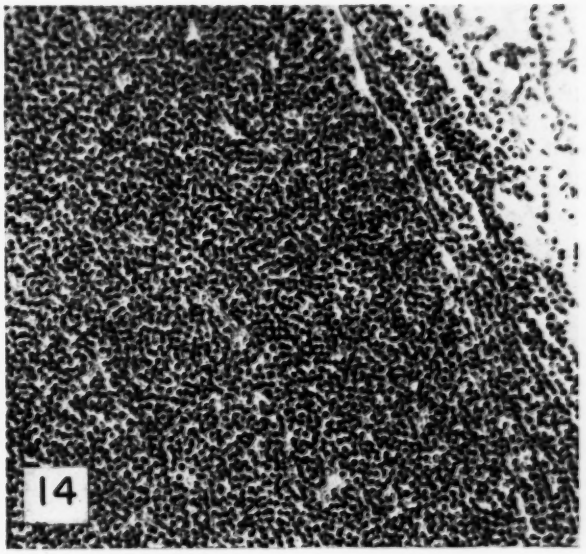


FIG. 11.—Papillary cyst adenocarcinoma from an A × C female rat, 348 days after it received 4 mgm. of diethylstilbestrol in a cholesterol pellet. Mag. × 150.

FIG. 12.—Mammary carcinoma from a Fischer line 344 female rat, 302 days after it received 7 mgm. of diethylstilbestrol in a cholesterol pellet. Mag. × 125.

FIG. 13.—Adenoacanthoma from an A × C female rat, 349 days after it received 4 mgm. of diethylstilbestrol in a cholesterol pellet. Mag. × 50.

FIG. 14.—Lymphosarcoma from an A × C female rat, 322 days after it received 4 mgm. of diethylstilbestrol in a cholesterol pellet. Mag. × 125.

with metastases to the lung and lymph nodes, which may have been related to the previous estrogenic stimulation. Another A × C female had a mixed tumor (chondrosarcoma and adenocarcinoma) of the ovary and a third, a carcinoma of the adrenal cortex. The latter probably resulted from the treatment, as it is a common sequel to estrogenic treatment in another strain of rats. Two A × C females had benign tumors of the thymus and 1 a lymphosarcoma of the ileocolic mesentery, which were probably unrelated to the treatment, since they occur in untreated rats of this strain.

Two Fischer line 344 males had adenoma of the adrenal cortex, 1 an adenocarcinoma, and another had a hepatoma. A lymphosarcoma of the ileocolic mesentery was observed in 1 Fischer male, and a Fischer line 344 female had a squamous carcinoma

of the left ear, which were probably unrelated to the treatment.

Aside from the bladder carcinomas previously mentioned in the Copenhagen line 2331 rats, 1 female had a myosarcoma of the uterus; 1 male, a benign tumor of the thymus; 1 male, a lymphosarcoma of the ileocolic mesentery; and 1 male, a lymphocytic leukemia. The 3 last-mentioned neoplasms were probably unrelated to the treatment, although leukemia and lymphosarcoma are rare in untreated rats of this line.

DISCUSSION

Comparison of the results of Series I and II show that a slow continuous absorption of diethylstilbestrol is an effective mammary cancer incitant in some strains of rats, whereas a cyclical or more rapid absorption of even larger doses with a short intervening rest period is ineffective in rats of the same inbred line. Only 5 A × C line 9935 females and 3 A × C line 9935 males of Series I survived as long as the latent period previous to the observation of the first gross tumors in A × C rats of Series II, but no tumors could be identified by microscopic examination of their mammary tissue. These rats had absorbed from 20 to 39.1 mgm. of diethylstilbestrol. In a subsequent series, 4 A × C line 9935 rats survived for a year or more after the implantation of a single 20 to 25 mgm. pellet of crystalline diethylstilbestrol, plus 2 per cent methyl cellulose used as a binder without developing any mammary tumors.

The difference in response between the rats of the Copenhagen line 2331 and those of the other 2 lines that were tested, may be related to the manner of excreting the diethylstilbestrol and the formation of urinary calculi, or to the pre-existing disturbance in metabolism. However, the physiological processes resulting in formation of bladder calculi and bladder neoplasms in one line and mammary neoplasms in the other two are not mutually exclusive, as shown in Fig. 2, by 4 A × C rats with mammary cancer and bladder calculi. The mammary tissue of the Copenhagen rats of both series was less well developed than that of the rats of the two other lines, but areas of hyperplasia with lactating dilated ducts were commonly found and a few lesions which could be considered as precancerous were also observed. The mammary tissue of the Fischer line 344 rats seemed to be even more copiously developed than that in the A × C rats which responded with the higher percentage of mammary cancers. Possibly another estrogen or dose might reverse the reactions of these rats. For α-estradiol (17), the Fischer line 344 rats showed the highest threshold for vaginal estrus and an impaired ability for hepatic estrogenic inactivation, while the Copenhagen line 2331 rats exhibited a high threshold for vaginal estrus and the greatest ability for hepatic inactivation. The incomplete data on the estrone treatment of rats of these three strains, however, indicate a response similar to diethylstilbestrol. The observations recorded here clearly indicate constitutional differences in response to diethylstilbestrol and in the incidence of mammary gland and bladder cancer in the rat. That these variations are dependent upon genetically determined physiological differences in the absorption, utilization or excretion of diethylstilbestrol, rather than gene differences specifically related to cancer susceptibility, is also indicated.

SUMMARY

1. Pellets of compressed cystalline diethylstilbestrol weighing 15 to 25 mgm. were implanted in the scapular region of 30 rats of both sexes of each of 3 distinct inbred lines and were replaced individually as soon as the previous pellet was known to be completely absorbed.

2. The rats of the 3 lines varied in survival and in the absorption rate of the diethylstilbestrol. Rats of Fischer line 344 succumbed first after the most rapid absorption of the hormone. Females of this line lived an average of only 67 days and absorbed an average of 19 mgm. per rat. The males survived an average of 136 days and absorbed 29 mgm. of diethylstilbestrol per rat. The Copenhagen line 2331 males survived for 289 days and the females for 200 days, having absorbed an average of 38 and 30 mgm. of hormone, respectively. The rats of A × C line 9935 were intermediate; the males survived for 167 days and the females for 186 days, in which time the average absorption of diethylstilbestrol was 24 and 25 mgm. per rat.

3. The most conspicuous pathological lesions included pituitary adenomas, fatty livers, and bladder calculi and papillomas. Pronounced strain differences were observed in the expression of these lesions. The pituitaries in the Fischer rats averaged 300 and 400 mgm. respectively in males and females, and only 98 and 61 mgm. in Copenhagen males and females, and were intermediate in the A × C rats. Fatty livers were confined to Fischer rats, and with 3 exceptions, the bladder calculi and papillomas were confined to Copenhagen male rats.

4. Pellets of 75 per cent cholestrol containing 4 to 15 mgm. of diethylstilbestrol were implanted in the scapular region of a second and similar series of

rats and no reimplantations were necessary to keep the rats in a constant state of hyperestrinism for the remainder of their lives.

5. The average survival period for the Fischer line 344 rats was increased to 200 days for the females and 367 days for the males with the more slowly absorbed diethylstilbestrol. The A × C males lived an average of 388 days and the females 442 days whereas the Copenhagen males and females survived 528 and 499 days, respectively.

6. Mammary cancer was observed in 17 or 80 per cent of the A × C males, in 22 or 85 per cent of the A × C females. Only 1 or 17 per cent of the Fischer line 344 females and 5 or 22 per cent of the Fischer line 344 males had mammary cancer.

7. No mammary cancers were observed in treated male or female Copenhagen line 2331 rats but 16 or 62 per cent of the males and 6 or 29 per cent of the females had either bladder papillomas or squamous cell cancer associated with urinary calculi.

8. The mammary cancers induced in Fischer line 344 and A × C line 9935 rats by slowly absorbed diethylstilbestrol included 111 adenocarcinomas, 11 adenocarcinomas and squamous cell cancers or adenoacanthomas, 4 solid carcinomas, 2 solid carcinomas with papillary areas, 1 interductal carcinoma and 4 that were unclassified.

REFERENCES

1. Benjamin, J. A., Wilson, J. G., and Leahy, Alice D. Study of Experimental Urinary Calculi: II. Quantitative Microchemical, Spectrographic, and Citric Acid Analyses of Albino Rat Calculi with a Preliminary Apatite Report. J. Urol., **54**:516-524. 1945.
2. Bittner, J. J. Some Possible Effects of Nursing on the Mammary Gland Tumor Incidence in Mice. Science, **84**:162-163. 1936.
3. Bittner, J. J. Breast Cancer in Mice as Influenced by Nursing. J. Nat. Cancer Inst., **1**:155-168. 1940.
4. Bittner, J. J. The Milk-Influence of Breast Tumors in Mice. Science, **95**:462-463. 1942.
5. Curtis, M. R., Bullock, F. D., and Dunning, W. F. A Statistical Study of the Occurrence of Spontaneous Tumors in a Large Colony of Rats. Am. J. Cancer, **15**:67-121. 1931.
6. Eisen, M. J. The Occurrence of Benign and Malignant Mammary Lesions in Rats Treated with Crystalline Estrogen. Cancer Research, **2**:632-643. 1942.
7. Gardner, W. U. Estrogens in Carcinogenesis. Arch. Path., **27**:138-170. 1939.
8. Geschickter, C. F., and Byrnes, Elizabeth W. Factors Influencing the Development and Time of Appearance of Mammary Cancer in the Rat in Response to Estrogen. Arch. Path., **33**:334-356. 1942.
9. Green, R. G., Moosey, M. M., and Bittner, J. J. Antigenic Character of the Cancer Milk Agent in Mice. Proc. Soc. Exper. Biol. & Med., **61**:115-117. 1946.
10. Lacassagne, A. Apparition de cancers de la mamelle chez la souris mâle, soumise à des injections de folliculine. Compt. rend. Acad. d. sc., **195**:630-632. 1932.
11. Lacassagne, A. Influence d'un facteur familial dans la production, par la folliculine, de cancers mammaires chez la souris mâle. Compt. rend. Soc. de biol., **114**:427-429. 1933.
12. Korteweg, R. On Manner in Which Disposition to Carcinoma of Mammary Gland is Inherited in Mice. Genetica, **18**:350-371. 1936.
13. McEuen, C. S. Occurrence of Cancer in Rats Treated with Oestrone. Am. J. Cancer, **34**:184-195. 1938.
14. Nelson, W. O. The Induction of Mammary Carcinoma in the Rat. Yale J. Biol. & Med., **17**:217-228. 1944.
15. Schenken, J. R., Burns, E. L., and McCord, W. M. Occurrence of Urinary Calculi in Inbred Strain (C_3H) of Mice Treated with Estrogen. Endocrinology, **30**:344-352. 1942.
16. Segaloff, A., and Dunning, W. F. The Effect of Strain, Estrogen, and Dosage on the Reaction of the Rat's Pituitary and Adrenal to Estrogenic Stimulation. Endocrinology, **36**:238-240. 1945.
17. Segaloff, A., and Dunning, Wilhelmina F. The Inactivation of α-Estradiol by Inbred Rats. Endocrinology, **39**:289-292. 1946.
18. Staff of Roscoe B. Jackson Memorial Laboratory. The Existence of Non-Chromosomal Influence in the Incidence of Mammary Tumors in Mice. Science, **78**:465-466. 1933.
19. Wilson, J. G., Benjamin, J. A., and Leahy, Alice D. Study of Experimental Urinary Calculi: I. Methods for Producing and Preventing Calculous Formation in the Bladder and Urethra of Albino Rats. J. Urol., **54**:503-515. 1946.

Partial Separation of the Mammary Tumor Milk Agent and a Comparison of Various Sources of the Agent*

Cyrus P. Barnum, Ph. D., Zelda B. Ball, Ph. D., and John J. Bittner, Ph. D.

(From the Department of Physiological Chemistry and the Division of Cancer Biology of the Department of Physiology, University of Minnesota Medical School, Minneapolis 14, Minnesota)

(Received for publication February 10, 1947)

The spontaneous development of mammary tumors in mice is dependent mainly upon three factors (3): genetic susceptibility, hormones, and the milk agent, which the young obtain from their mothers during nursing.

The milk agent has been shown to pass through bacterial filters (1, 3, 4), to be destroyed at 60° C. (1, 2), to be stable for 1 to 2 hours at pH values between 5 and 10 (2), to survive through 10 transplant generations in mice that did not originally contain the agent (7), and to survive (or proliferate) when carried as a cell-free filtrate through 9 passages in eggs (7, 10). Furthermore, it has been shown that it can be sedimented from extracts of mammary glands at 110,000 times gravity (20) and from mouse milk at 60,000 times gravity (11). In these experiments the agent was assayed in test animals by injecting or feeding fractions obtained from 0.2 cc. of milk or from 0.33 to 1 gm. of mammary tumor tissue or lactating glands. More recent work (8) has shown that the agent can be demonstrated in an extract of lactating mammary glands representing 2×10^{-4} gm. of gland tissue. The presence of the agent has also been demonstrated in whole blood, spleen and thymus (5), and in liver (10).

The present work was undertaken in an effort to study more quantitatively the cytoplasmic distribution of the agent by combining the technics of differential centrifugation with those of serial dilution, and also, to attempt to find the most potent source of the agent.

MATERIALS AND METHODS

The test animals used were ABC females between 3 and 6 weeks of age obtained as follows:

C57 Black ♀ × A ♂
|
BAF_1 ♀ × A ♂
|
ABC

The tumors or lactating glands used as sources of the agent were ground in a mortar with distilled water or saline-buffer (0.01 *M* phosphate at pH 7.6 in 0.85 per cent NaCl) and following centrifugation the various fractions were resuspended in saline-buffer and injected intraperitoneally or subcutaneously. The fractions were injected in amounts ranging from 10^{-2} to 10^{-7} gm. equivalents; that is, the amount of a given fraction that was derived from 10^{-5} gm. of original wet tissue is referred to as 10^{-5} gm. equivalents. Following injection the animals were force-bred for at least 2 or 3 litters and then were allowed to breed normally. The animals average 4 or 5 litters apiece. The experiments were terminated when the animals were 2 years of age and the surviving mice were autopsied. With only one exception, for all of the experiments reported in this paper the tumors were established as mammary gland carcinoma by microscopic examination.[1]

In all experiments, particularly the fractionation experiments in which preparation required up to 8 hours, the utmost care was taken to maintain the temperature of the material as close to 0° C. as possible. The tissues were harvested into a beaker in an ice-water bath, macerated by forcing through a tissue press with holes about 1 mm. in diameter, ground in a cold mortar in an ice-water bath, and centrifuged in the cold. The initial centrifugation was carried out in a Sorvall angle centrifuge operated in the refrigerator. The next two centrifugations were done in an International centrifuge equipped with a multispeed attachment. This centrifuge, which develops a force of 23,000 × g. at the center of the 14 cc. plastic tubes that were used, was operated in a cold room at about −15° C., and the material was kept just above its freezing point by adjusting the cover of the centrifuge. The final centrifugation was carried out at 70,000

* This work was aided by grants from the Graduate School Cancer Research Fund of the University of Minnesota, The Jane Coffin Childs Memorial Fund for Medical Research, and The Donner Foundation, Incorporated.

[1]The authors are indebted to Dr. R. A. Huseby and Mr. Norman Kretchmer for their help in diagnosing the tumors.

× g. in an air-driven ultra-centrifuge.[2] The head of this centrifuge was chilled prior to running, and since it operates in a vacuum, the material remained quite cool throughout the run. The final fractions were always diluted in cold saline-buffer just prior to injection, and for a given fraction the most dilute solution was always injected first and the most concentrated one last. The longest time interval between removal of the tissue and injection of a fraction was in the first fractionation experiment, in which 10 hours elapsed. This particular fraction gave a good incidence of tumors.

RESULTS

In this series of experiments the first attempt to use dilute solutions for testing the activity of the agent was one in which spontaneous tumors arising in A strain mice were used as the source material. The tumors were macerated and ground in a mortar with sand and 9 volumes of distilled water. The extract was cleared of debris by spinning at about 500 × g. for 20 minutes, and the supernatant fluid was removed from under the small amount of fat on the surface. The results of injecting this supernatant fluid into test animals in 10^{-1} and 10^{-3} gm. equivalent amounts are shown in Table I.

TABLE I: ACTIVITY OF THE MILK AGENT IN EXTRACTS OF SPONTANEOUS TUMOR TISSUE

Gram. equiv. injected	Mice living to 1st tumor	No. of mice with tumors	% with tumors	Av. tumor age, days
10^{-1}	15	12	80	307
10^{-3}	15	11	73	326

Two large scale experiments were set up involving a combination of serial dilution and fractionation by differential centrifugation. The first of these was designed largely upon the centrifugation technic described by Claude (12, 13). Spontaneous tumors arising in A strain mice were put through the tissue press and ground in a cold mortar with the slow addition of 4 volumes of cold saline-buffer. The resulting suspension was centrifuged in the refrigerator at 500 × g. for 5 minutes and the supernatant fluid decanted and spun again for 5 minutes at this speed. This supernatant fluid, the cytoplasmic extract, was labelled E. Part of the extract was spun at 23,000 × g. for 5 minutes. The resulting sediments were resuspended in saline-buffer, cleared at low speed for 1 minute, and the supernatant fluid again spun for 5 minutes at 23,000 × g. Once again the sediments were resuspended, cleared at low speed and resedimented at 23,000 × g. for 5 minutes. This time the sediments were resuspended and referred to as the large granule, or L, fraction.

The supernatant fluid from the first 5 minute run at 23,000 × g. was spun for 1 hour at 23,000 × g., and the resulting supernate, referred to as MS, was carefully removed with a syringe and needle from beneath the few fatty particles on the surface. The pellets were resuspended in saline-buffer and cleared of large aggregates by spinning for 5 minutes at 23,000 × g. A very large sediment appeared (*see* Discussion) but this was discarded and the slightly opalescent supernatant fluid was spun again for 1 hour at 23,000 × g. The pellets were again resuspended and this time spun for 1 minute at low speed and the supernate referred to as the microsome, or M, fraction.

The four fractions available for injection were E, L, M and MS. These were diluted just prior to injection into test animals. The results are shown in Table II.

TABLE II: ACTIVITY OF THE MILK AGENT IN CENTRIFUGAL FRACTIONS OF SPONTANEOUS MAMMARY CARCINOMA

Fraction	Gram. equiv. injected	Mice living to 1st tumor	No. of mice with tumors	Av. tumor age, days
E	10^{-6}	6	0	
	10^{-5}	8	0	
	10^{-4}	8	2	330
	10^{-3}	9	4	336
	10^{-2}	10	5	319
L	10^{-7}	9	0	
	10^{-6}	9	0	
	10^{-5}	7	0	
	10^{-4}	10	0	
	10^{-3}	9	3	417
	10^{-2}	8	3	389
M	10^{-7}	8	0	
	10^{-6}	7	0	
	10^{-5}	9	1	473
	10^{-4}	6	2	400
	10^{-3}	9	1	524
	10^{-2}	8	5	327
MS	10^{-6}	8	0	
	10^{-5}	9	0	
	10^{-4}	8	0	
	10^{-3}	10	0	
	10^{-2}	8	1	440

By pooling the 3 most concentrated dilutions of each fraction, it is possible to get larger and more significant groups as seen in Table III.

TABLE III: SUMMARY OF RESULTS WITH THE 3 MOST CONCENTRATED DILUTION OF EACH FRACTION

Fraction	Gram. equiv. injected	Mice living to 1st tumor	No. mice with tumors	% with tumors	Av. tumor age, days
E	10^{-2}–10^{-4}	27	11	41	327
L	10^{-2}–10^{-4}	27	6	22	403
M	10^{-2}–10^{-4}	23	8	35	370
MS	10^{-2}–10^{-4}	26	1	4	440

[2]We wish to thank the late Dr. R. G. Green and Miss Marye Moosey for the use and operation of the ultra-centrifuge.

The total nitrogen found in the amount of each fraction derived from 1 gm. of tumor tissue is indicated in Table IV.

TABLE IV: TOTAL NITROGEN VALUES FOR EACH FRACTION

Fraction	Mgm. *N* per gm. tissue
E	11.15
L	0.19
M	0.083
MS	9.05

In addition to the nitrogen determinations on these fractions, an attempt was made to estimate the ribose content, and it was evident that a large portion of the cytoplasmic ribose was still left up in the MS fraction. In an attempt to sediment some of this ribose-containing material, another experiment was set up in which 3 particulate fractions were isolated.

The technic was essentially the same as in the preceeding experiment. Tissue from spontaneous tumors in mice of the A strain was ground with saline-buffer, cleared of debris and the supernatant fluid labelled E. The L fraction was prepared as before, except that in place of the second low speed clearing run, the resuspended sediment was placed in a graduated cylinder and the heavy aggregates allowed to settle. The homogeneous supernate was carefully removed and labelled L.

In the case of the M fraction, the pellet was spun down from the supernatant fluid occurring after the first 5 minute spin at 23,000 × g., by spinning 1 hour at 23,000 × g. This pellet was resuspended, the heavy aggregates allowed to settle, and the opalescent dispersion carefully removed and labelled M.

The supernatant fluid after the 1 hour spin at 23,000 × g. was spun in the ultracentrifuge for 1 hour at 70,000 × g. The clear supernate was carefully removed from beneath the fat flakes on the surface and labelled S. The pellets were resuspended in saline-buffer, the heavy aggregates allowed to settle and the opalescent supernatant fluid removed and labelled U.

The results of injecting these 5 fractions are shown in Table V.

As may be seen, the U and S fractions produced a good incidence of tumors when 10^{-2} gram equivalents were injected, but very few tumors occurred from the higher dilutions. In comparing the fractions, it was felt most significant to pool the data from the middle 3 dilutions as shown in Table VI.

The analyses for nitrogen on these fractions are shown in Table VII.

At about the same time that these two fractionation experiments were made, we were interested in attempting to discover the best source material for isolating the agent. An experiment was arranged to compare transplanted tumor tissue, spontaneous tumor tissue, and lactating glands from animals containing the agent. The transplanted tissue used was derived from a spontaneous tumor arising in an A strain mouse and was carried for two generations in A strain mice. The spontaneous tumor tissue used was also from an A strain mouse. One set of lactating glands was obtained from an A strain mouse that was 7 months of age

TABLE V: ACTIVITY OF THE MILK AGENT IN CENTRIFUGAL FRACTIONS OF SPONTANEOUS MAMMARY CARCINOMA

Fraction	Gram. equiv. injected	Mice living to 1st tumor	No. of mice with tumors	Av. tumor age, days
E	10^{-6}	7	1	354
	10^{-5}	8	1	438
	10^{-4}	9	4	271
	10^{-3}	8	4	305
	10^{-2}	6	6	310
L	10^{-6}	7	0	
	10^{-5}	8	3	520
	10^{-4}	7	4	426
	10^{-3}	3	3	328
	10^{-2}	6	2	270
M	10^{-6}	5	0	
	10^{-5}	6	2	428
	10^{-4}	8	5	322
	10^{-3}	9	8	310
	10^{-2}	7	5	330
U	10^{-6}	7	0	
	10^{-5}	8	1	563
	10^{-4}	9	0	
	10^{-3}	5	2	550
	10^{-2}	9	5	417
S	10^{-6}	8	0	
	10^{-5}	8	1	618
	10^{-4}	5	0	
	10^{-3}	6	0	
	10^{-2}	6	5	333

TABLE VI: SUMMARY OF RESULTS OF TESTS OF ACTIVITY OF FRACTURES FROM POOLED DATA FOR MIDDLE 3 DILUTIONS

Fraction	Gram. equiv. injected	Mice living to 1st tumor	No. mice with tumors	% with tumors	Av. tumor age, days
E	10^{-3}–10^{-5}	25	9	36	305
L	10^{-3}–10^{-5}	18	10	56	425
M	10^{-3}–10^{-5}	23	15	65	330
U	10^{-3}–10^{-5}	22	3	14	554
S	10^{-3}–10^{-5}	19	1	5	618

TABLE VII: NITROGEN VALUES OF FRACTIONS

Fraction	Mgm. *N* per gm. tissue
E	11.43
L	0.27
M	0.79
U	0.90
S	8.03

and was nursing her second litter which had been born 17 days before. These glands contained a fairly large amount of milk despite the fact that the young had been left with the mother until the time of sacrifice. A second set of glands was obtained from a C3H or Z mouse that was 6 months of age and was nursing her second litter born 18 days before. Here again the young were left with the mother until the time of sacrifice, but in this case the glands contained no appreciable amount of milk.

The 4 tissues were ground separately in mortars with sand and saline-buffer and then cleared by spinning 6 minutes at 500 × g. The extracts were labelled T, S, A and Z from transplanted tumor tissue, spontaneous tumor tissue, A strain glands and Z strain glands respectively.

These extracts were diluted and injected into ABC mice as in the previous experiments. In the case of the extracts from the transplanted tumor tissue, the dilutions were injected either intraperitoneally or subcutaneously, but since there was no significant difference, the results are pooled. All other injections were given intraperitoneally. In a few of the mice injected subcutaneously with extracts of the transplanted tumor tissue, there occurred transplanted tumors at the site of the injection within a few weeks, so these animals were discarded. It is apparent that a few intact cells were included in the supernate decanted after spinning 6 minutes at 500 × g. The present practice in this laboratory is to spin 4 minutes at 1500 × g. This brings down all intact cells and all or most of the nuclei. The extracts from some tissues must be spun again to clear the remaining nuclei.

The results of injecting these extracts of tumors and mammary glands are shown in Table VIII.

Here it seemed most significant to pool the data from the 3 highest dilutions as has been done in Table IX.

TABLE VIII: ACTIVITY OF THE MILK AGENT IN EXTRACTS OF VARIOUS TISSUES

Fraction	Gram. equiv. injected	Mice living to 1st tumor	No. of mice with tumors	Av. tumor age, days
T	10^{-6}	12	1	371
	10^{-5}	15	3	507
	10^{-4}	13	2	327
	10^{-3}	10	1	184
	10^{-2}	8	4	351
S	10^{-6}	6	2	395
	10^{-5}	6	2	297
	10^{-4}	8	3	378
	10^{-3}	7	5	319
	10^{-2}	8	7	294
A	10^{-6}	7	3	294
	10^{-5}	6	6	293
	10^{-4}	8	8	349
	10^{-3}	6	4	314
	10^{-2}	6	3	330
Z	10^{-6}	6	1	352
	10^{-5}	7	3	329
	10^{-4}	8	5	364
	10^{-3}	6	5	318
	10^{-2}	6	6	352

TABLE IX: SUMMARY OF RESULTS FROM POOLED DATA OF THE THREE HIGHEST DILUTION OF FRACTIONS

Fraction	Gram. equiv. injected	Mice living to 1st tumor	No. mice with tumors	% with tumors	Av. tumor age, days
T	10^{-4}–10^{-6}	40	6	15	424
S	10^{-4}–10^{-6}	20	7	35	360
A	10^{-4}–10^{-6}	21	17	81	319
Z	10^{-4}–10^{-6}	21	9	43	351

DISCUSSION

In analyzing the data reported here, and in attempting to correlate it with previous work in this and other laboratories two important facts stand out. The first is that in fractionation experiments based on injecting relatively concentrated preparations of the various fractions, the interpretation may be very misleading since 10^{-6} gm. of tissue have been shown to contain enough agent to induce tumors. This is brought out very clearly in Table V where the U and S fractions showed a good incidence of tumors when 10^{-2} gm. equivalents were administered, but gave rise to only occasional tumors which arose much later in life when smaller amounts were used. Had 10^{-2} gm. equivalents been the only solution injected, the interpretation might well have been that the agent was equally distributed throughout the cytoplasm, that is, that L, M, U and S all showed the same activity. The interpretation may be even more misleading when one considers that in some instances dilution of an extract up to a certain point may actually give rise to a higher incidence of tumors (8).

A previous fractionation experiment, the preliminary results of which have been reported (2), further illustrates the difficulty of interpreting data when concentrated solutions are used. The final data on this experiment have not altered the previous conclusions, and show that when an extract of spontaneous tumor tissue was brought to a pH of 5.5 the resulting precipitate and supernatant fluid gave rise to roughly the same incidence of tumors when injected in 1 gm. equivalent amounts into small groups of test animals. Also, in this same experiment extracts of the tumor tissue were treated with the basic protein, salmine, and again there did not appear to be a very clear-cut separation of the agent as determined by in-

jecting 1 gm. equivalents of precipitate and supernatant fluid into test animals. Certainly part of the difficulty in interpreting such a fractionation experiment is due to the tremendous doses used, and it is possible that serial dilution might have shown one fraction to be much more active than another. This is unlikely in this particular fractionation experiment, however, since the major portion of the agent is now known to be associated with the large granule and microsome fractions of the tumor cell cytoplasm as seen in Tables II and V. Claude (13) has shown that these fractions from liver cells are maximally precipitated at a pH of 3.5, but that precipitation is not complete at pH 5.5. Furthermore, unpublished work in this laboratory has shown that the concentration of salmine used in the above experiment (0.1 per cent) precipitates only about 30 per cent of the microsome fraction from a cytoplasmic extract of tumor tissue, though the same concentration of salmine will almost completely precipitate a suspension of purified microsomes. It would seem probable, therefore, that the agent was not separated in any clear cut manner either by 0.1 per cent salmine or at pH 5.5; but such a conclusion would not be justified from the data on the incidence of tumors, even though this incidence were identical in two fractions, since only one concentration of each fraction was injected.

A second observation that seems important for future work has been that when transplanted tumor tissue was used as the source of the agent, the results were extremely erratic. In one experiment, for example, an extract of transplanted tumor tissue was injected into 130 mice in amounts ranging from 10^{-2} to 10^{-6} gm. equivalents. Only 25 of these mice developed tumors, but the surprising fact was that these tumors were rather equally distributed among all animals that received diluted material in the series. A comparable situation is seen in Tables VIII and IX, where the transplanted tumor tissue has given rise to much the lowest incidence of tumors but still there are tumors occurring after injection of 10^{-5} and 10^{-6} gm. equivalents of the materials tested. No adequate explanation is apparent for this behavior, but it does indicate that transplanted tumor tissue is a much less reliable source of the agent for experimental work than are, for example, mammary glands.

From the data reported in Tables VIII and IX it is apparent that the lactating glands of the A strain mouse used in this experiment were an entremely potent source of the agent. The dilutions must be carried further since 10^{-6} gm. equivalents still gave rise to a significant incidence of tumors. It must be pointed out that these were glands from a single mouse, as was true also for the C3H or Z gland tissue used in this experiment, and consequently the somewhat lower incidence of tumors arising from the extract of the Z glands cannot be definitely interpreted as a strain difference but may be merely an individual difference.

The available history on the A strain mouse whose glands were used shows that the mother and one of two daughters died of mammary cancer. The second daughter was an experimental animal that had been placed on a calorie-restricted diet for which the incidence of cancer was very low. In the case of the Z strain mouse the mother and 3 of 4 sisters died of mammary cancer. This possibility of a strain difference should be tested further at higher dilutions from extracts obtained from the pooled glands of many mice of the two strains. In comparing the relative activity of the milk agent in the glands of A and Z strain mice, the test animal should also be taken into consideration. It is possible, for example, that the agent from glands of Z strain mice might show a greater activity if tested in ZBC mice. However, it is interesting to note that these present results, which indicate possibly a greater activity of the agent from A strain mice, are in accord with the findings on reciprocal crosses of A and Z mice (6, 9). In this work the first and second generation hybrids with A mothers and Z fathers (AZF_1 and AZF_2) showed a significantly higher incidence of tumors than the reciprocal crosses (ZAF_1 and ZAF_2) that had Z mothers. In as much as the genetic constitution of the two crosses should be identical, it seemed most plausible to ascribe the differences in incidence to differences in the concentration and/or activity of the milk agent in the milk of the A and Z mothers.

Since the agent is extremely potent in lactating mammary glands but apparently much less potent in blood (10), it seems unnecessary to postulate that its occurrence in milk may be due to the presence of lymphoid elements containing the agent as suggested by Gardner (14, 15).

As seen in Tables VIII and IX, the transplanted tumor tissue used appeared to be the least potent source of the agent. Again this tissue was derived from a single spontaneous tumor that was carried for two generations in A strain mice, that is, in mice who themselves contained the agent. However, the agent has been demonstrated (7) in transplanted tumor tissue carried for 10 generations in mice that did not themselves contain the agent.

The tissue from spontaneous tumors that was used in this experiment and in the two fractiona-

tion experiments appeared to be a fairly good source of the agent though probably not as good as lactating glands from mice of the A stock. Again it should be pointed out that, while the present work requires extension, the indication that it gives of a greater activity of the agent in lactating glands of the A strain mouse was obtained only because dilutions were carried out to 10^{-6} gm. equivalents. Had these dilutions been only from 10^{-2} to 10^{-4} gm. equivalents then, as seen from Table VIII, the incidence of tumors would have been as great from spontaneous tumor tissue and from glands of the Z strain mouse as from the glands of the A strain mouse.

The fractionation experiments shown in Tables II, III, V and VI indicate that only a very small fraction of the agent, probably less than 1 per cent, remains in the supernatant fluid after spinning extracts of spontaneous tumor tissue at 23,000 × g. for 1 hour. The differences between the two larger particulate fractions, the large granules and the microsomes, are not nearly so clear-cut. There is a slight indication, based largely on average tumor age, that the agent may be more active in the microsome fraction, but this is by no means certain and will require further work.

The large granule and microsome fractions present a very distinct difference in appearance in the sedimented states. The large granules form a fairly well-packed, tan, opaque sediment, while the microsomes give a tightly packed, light-amber pellet that is perfectly transparent except for a very small, whitish star at the bottom. With improvement of technics it is now possible to resuspend both of these fractions to such a state that a smaller portion is lost when they are cleared at lower speeds. However, subsequent work with liver and with tumor tissue has led us to the conclusion that it is not necessary to clear the resuspended microsomes, for example, by a 5 minute spin at 23,000 × g., since it appears that all of the large granules (and probably some microsomes) are brought down by the initial 5 minute spin at 23,000 × g. As seen by comparing Tables IV and VII, the clearing run in the first experiment brought about the loss of about 90 per cent of the microsome fraction. The present practice in this laboratory, therefore, is to resuspend and then resediment these fractions without employing any low speed clearing runs.

In the two fractionation experiments Bial's orcinol-HCl test (17) was employed in an attempt to quantitate the ribose in the various fractions. The ribose, as found by this test, in an MS fraction was approximately equally distributed between the U and S fractions. Subsequent work, employing the Beckman ultraviolet spectrophotometer and the technics of Schneider for separation of nucleic acids (18) has shown that the major portion of the ribose of the particulate fractions (L, M and U) is present as nucleic acid. This is true whether these fractions are derived from liver, tumor tissue, mammary glands containing the agent, or mammary glands without the agent. Consequently, the finding of nucleic acid in a fraction that contains the agent cannot be cited as evidence that the agent is a nucleoprotein complex, as Shimkin (19) has interpreted the results obtained by Kahler and Bryan (16).

SUMMARY

By employing the technics of serial dilution, A strain lactating mammary glands have been shown to be an extremely potent source of the mammary tumor milk agent. Extracts give rise to tumors even at a million-fold dilution. C3H or Z strain lactating glands and spontaneous tumor tissue appear to be a less potent source of the agent, while transplanted tumor tissue seems to be a relatively poor and unreliable source.

The agent can be essentially completely sedimented from an extract of spontaneous tumor tissue by centrifuging for 1 hour at 23,000 × g. The agent has been found in both the large granule ("mitochondria") fraction and in the microsome fraction of a cytoplasmic extract of spontaneous tumor tissue.

REFERENCES

1. Andervont, H. B., and Bryan, W. R. Properties of the Mouse Mammary-Tumor Agent. J. Nat. Cancer Inst., **5**:143-149. 1944.
2. Barnum, C. P., Ball, Zelda, B., Bittner, J. J., and Visscher, M. B. The Milk Agent in Spontaneous Mammary Carcinoma. Science, **100**:575-576. 1944.
3. Bittner, J. J. Possible Relationship of the Estrogenic Hormones, Genetic Susceptibility, and Milk Influence in the Production of Mammary Cancer in Mice. Cancer Research, **2**:710-721. 1942.
4. Bittner, J. J. The Milk-Influence of Breast Tumors in Mice. Science, **95**:462-463. 1942.
5. Bittner, J. J. Inciting Influence in the Etiology of Mammary Cancer in Mice. A. A. A. S. Research Conference on Cancer, 1944, pp. 63-96.
6. Bittner, J. J., Huseby, R. A., Visscher, M. B., Ball, Zelda B., and Smith, Fern. Mammary Cancer and Mammary Structure in Inbred Stocks of Mice and Their Hybrids. Science, **99**:83-85. 1944.
7. Bittner, J. J., Evans, C. A., and Green, R. G. Survival of the Mammary Tumor Milk Agent of Mice. Science, **101**:95-97. 1945.
8. Bittner, J. J. Characteristics of the Mammary Tumor Milk Agent in Serial Dilution and Blood Studies. Proc. Soc. Exper. Biol. & Med., **59**:43-44. 1945.

9. Bittner, J. J., and Huseby, R. A. Relationship of the Inherited Susceptibility and the Inherited Hormonal Influence in the Development of Mammary Cancer in Mice. Cancer Research, **6**:235-239. 1946.
10. Bittner, J. J. Unpublished data.
11. Bryan, W. R., Kahler, H., Shimkin, M. B., and Andervont, H. B. Extraction and Ultracentrifugation of Mammary Tumor Incitor of Mice. J. Nat. Cancer Inst., **2**:451-455. 1942.
12. Claude, A., and Fullam, E. F. An Electron Microscope Study of Isolated Mitochondria. Method and Preliminary Results. J. Exper. Med., **81**:51-62. 1945.
13. Claude, A. Fractionation of Mammalian Liver Cells by Differential Centrifugation. I. Problems, Methods, and Preparation of Extract. II. Experimental Procedures and Results. J. Exper. Med., **84**: 51-59; 60-89. 1946.
15. Gardner, W. U. Steroid Hormones in the Induction of Cancer. A. A. A. S. Research Conference on Cancer, 1946. Abstract in Cancer Research, **7**: 37-38. 1947.
16. Kahler, H., and Bryan, W. R. Ultracentrifugal Studies of Some Complexes Obtained from Mouse Milk, Mammary Tumor, and Other Tissues. J. Nat. Cancer Inst., **4**:37-45. 1943.
17. Mejbaum, Wanda. Über die Bestimmung kleiner Pentosemengen, insbesondere in Derivaten der Adenylsäure. Ztschr. physiol. Chem., **258**:117-120. 1939.
18. Schneider, W. C. Phosphorus Compounds in Animal Tissues. I. Extraction and Estimation of Desoxypentose Nucleic Acid and of Pentose Nuleic Acid. J. Biol. Chem., **161**:293-303. 1945.
19. Shimkin, M. B. A. A. A. S. Symposium No. 22. Mammary Tumors in Mice. 1945. pp. 209-222.
20. Visscher, M. B., Green, R. G., and Bittner, J. J. Characterization of the Milk Influence in Spontaneous Mammary Carcinoma. Proc. Soc. Exper. Biol. & Med., **49**:94-96. 1942.

The Response of Mice to the Simultaneous Application of Two Different Carcinogenic Agents

Werner G. Jaffé, Ph. D.

(*From the Instituto Quimio-Biologico, Caracas, Venezuela*)

(Received for publication February 4, 1947)

In a previous paper we reported on results of experiments in which rats were treated with two differently acting carcinogens (2). That study was made in order to investigate the possible influence of one such agent on the action of another. No interaction between the carcinogens was found. The present report deals with similar experiments on albino mice.

MATERIALS AND METHODS

Animals used in these experiments were albino mice of our own breed, 6 to 8 weeks old. The spontaneous tumor rate in this stock is 0.5 per cent at the age of 1 year if lung tumors are not included. The latter have been observed in 5 per cent of the animals autopsied at the age of 4 months, in 15 per cent of mice 6 months old and in 25 per cent of mice at 1 year of age.

The methylcholanthrene was dissolved in 0.1 ml. of olive oil and injected subcutaneously. A single injection was given to each animal. Urethane and *p*-dimethylaminoazobenzene were mixed with the diets in the amounts indicated in Table I and in the same manner as previously described (2). The carcinogenic diet was given from the day of injection of methylcholanthrene through the whole experimental period. At the end of 4 months the animals were killed and autopsied and examined for local tumors. The lungs were fixed in Bouin solution and formol as previously described (3) and examined for the presence of adenomas.

TABLE I: LOCAL AND LUNG TUMORS OBSERVED IN MICE 4 MONTHS AFTER BEGINNING OF TREATMENT

Treatment	Effective total	Local tumors %	Lung tumors, %
0.9 mgm. MC* subcu. + diet cont'g. 0.75% ethyl urethane	28	87	89
0.45 mgm. MC subcu. + diet cont'g. 0.15% ethyl urethane	75	76	95
0.225 mgm. MC subcu. + diet cont'g. 0.15% ethyl urethane	27	46	96
0.45 mgm. MC subcu. + diet cont'g. 0.015% *p*-dimethylaminoazobenzene	31	74	23
0.225 mgm. MC subcu. + diet cont'g. 0.015% *p*-dimethylaminoazobenzene	23	39	43
CONTROLS			
0.9 mgm. MC subcu.	164	80	45
0.45 mgm. MC subcu.	78	67	32
0.225 mgm. subcu.	68	41	31
Diet cont'g. 0.75% ethyl urethane	50	—	98
Diet cont'g. 0.15% ethyl urethane	14	—	100
Diet cont'g. 0.015% *p*-dimethylaminoazobenzene	22	—	25

* MC = methylcholanthrene

RESULTS

In Table I, a summary of the animals with local tumors and with pulmonary adenomas is given. In no case was a significant difference observed between the tumor incidence in the groups treated with 2 carcinogens as compared with the control groups treated with only 1 of the carcinogens.

The treatment with *p*-dimethylaminoazobenzene never caused detectable liver damage under the experimental conditions. Nevertheless, the dye, when fed with the diet, can be considered to be carcinogenic for the strain of mice used, for the data in Table II show that a significant rise in the rate of pulmonary adenomas over the spontaneous rate can be observed after 75 days of feeding the carcinogenic dye. Andervont, White and Edwards (1) have shown that *o*-aminoazotoluene produces lung tumors when fed to mice, but they did not observe such an effect with *p*-dimethylaminoazobenzene in their experiments with strain C mice.

TABLE II: LUNG TUMORS OBSERVED IN MICE 75 DAYS AFTER BEGINNING OF FEEDING ETHYL URETHANE OR *p*-DIMETHYLAMINOAZOBENZENE

Treatment	Effective total	Lung tumors, %
Diet cont'g. 0.015% *p*-dimethylaminoazobenzene	50	22
Diet cont'g. 0.15% ethyl urethane	75	59
Controls	57	5

* Present address: Department of Chemistry, Ministerio de Agricultura y Cria, Caracas, Venezuela, S. A.

DISCUSSION

The results of experiments with mice confirm those obtained previously with rats (2), namely that *p*-dimethylaminoazobenzene or ethyl urethane do not influence the number of tumors elicited by methylcholanthrene. The number of mice developing local tumors after a subcutaneous injection of methylcholanthrene was the same whether the animals received simutaneously another carcinogen with the diet or whether they were kept on the stock diet without further treatment.

Under the experimental conditions it was not possible to decide whether the action of injected methylcholanthrene in producing pulmonary adenomas in mice is additive to the action of urethane as the number of animals developing this kind of tumor was close to 100 per cent. In 2 series treated with methylcholanthrene and fed a diet containing *p*-dimethylaminoazobenzene no such additive action could be detected but the number of mice was not sufficient to draw definite conclusions.

The results indicate that in general 2 different carcinogens act independently. This observation does not necessarily exclude the possibility that under special conditions an interaction may be observed. It is known that carcinogenic hydrocarbons may have a retarding action on growth of transplanted tumors (7) and recently Murphy and Sturm (6) found that ethyl urethane inhibits the development of lymphatic tumors and leukemia in rats. This compound has a definite carcinogenic action in rats (4).

Indeed, we found recently that mice that had received *p*-dimethylaminoazobenzene with the diet for 6 weeks previous to a single injection of urethane developed fewer pulmonary adenomas than did the controls (5). Therefore care should be taken not to accept the independent action of simultaneously applied differently acting carcinogens as a rule without having studied each special case.

The present experiments show that feeding *p*-dimethylaminoazobenzene at a level of only 0.015 per cent for 75 days raises the spontaneous rate of pulmonary adenomas to a significant degree. No such action of the azo dye has been reported previously as far as we know, although the related compound, *o*-aminoazobenzene, has been shown to stimulate the development of pulmonary tumors in mice (1).

SUMMARY

Albino mice were injected subcutaneously with methylcholanthrene and were given a diet containing sufficient ethyl urethrane to produce pulmonary adenomas in nearly 100 per cent of the animals during the experimental period of 4 months. No difference in the number of animals developing local tumors was observed as compared with the controls. *p*-Dimethylaminoazobenzene fed in the diet also did not influence the number of local tumors elicited by methylcholanthrene.

Feeding of a diet containing *p*-dimethylaminoazobenzene alone augmented the number of mice bearing pulmonary adenomas to a significant degree over the spontaneous rate, while no liver damage could be detected under the conditions observed.

REFERENCES

1. Andervont, H. B., White, J., and Edwards, J. E. the Effect of Two Azo Compounds when Added to the Diet of Mice. J. Nat. Cancer Inst., **4**:583-586. 1944.
2. Jaffé, W. G. The Response of Rats to the Simultaneous Application of Two Different Carcinogenic Agents. Cancer Research, **7**:113-116. 1947.
3. Jaffé, W. G. Possible Linkage Between the Development of Local Tumors and Pulmonary Adenomas Induced by Methylcholanthrene in Non-Inbred Mice. Cancer Research, **7**:117-119. 1947.
4. Jaffé, W. G. Carcinogenic Action of Ethyl Urethane in Rats. Cancer Research, **7**:107-112. 1947.
5. Jaffé, W. G. Fenomenos inmunologicos en cancerologia. Rev. Policlinica, Caracas, **15**:1-12. 1946.
6. Murphy, J. B., and Sturm, E. The Effect of Urethane on Lymphatic Leukemia in Rats. Science, **104**:427. 1946.
7. Stern, K., and Willheim, R. The Biochemistry of Malignant Tumors. Brooklyn: Reference Press. 1943, pp. 191-194.

Studies On Drug Adsorption

Fixation of Quinine by Neoplastic and Non-Neoplastic Tissues*

F. E. Kelsey, Ph. D., and Alexander Brunschwig, M. D.

(*From the Departments of Pharmacology and Surgery, The University of Chicago 37, Illinois*)

(Received for publication April 9, 1947)

The observations reported in this paper were made in patients with a variety of malignant neoplasms. The purpose was to determine whether quinine might be selectively concentrated in such neoplasms. Previous studies on laboratory animals (1-3) revealed a striking species variation in quinine metabolism as well as an uneven distribution of quinine in the tissues and fluids of the body.

Many of the investigations on the biochemical variations between neoplastic and non-neoplastic tissues have concerned themselves with a direct analysis of tissue components and enzyme activities. It was hoped that the more indirect approach of observing the degree of concentration of drugs in such tissues might reveal quantitative differences that could eventually be used in designing a drug which would be specifically localized in neoplasms.

MATERIALS AND METHODS

Quinine, as the hydrochloride salt, was administered either by mouth or by vein from 2 to 7 hours preoperatively, to a selected group of patients with various types of neoplasms. Blood was drawn for analysis at the beginning and end of the operation, and the time of removal of the tumor and adjacent tissues noted. In all patients, the diagnosis was confirmed by histologic examination of the excised tissues.

Chemical procedures.—Analyses for quinine were done by a modification of a previously described method (1). Blood, plasma or tissue homogenate was dissolved by warming in 10 per cent K0H and after cooling was extracted by ethyl ether. An aliquot of the ether phase was extracted by shaking with 0.1 *N* H_2S0_4 and the fluorescence of the acid extract determined in a Coleman photofluorometer, model 12A, with B_1S and PC-1 filters. This method is sensitive to 0.1 μgm. Control analyses on tissues from patients who had received no drug gave values ranging from 0.1 to 0.4 μgm. of "apparent quinine" per gram of tissue.

Total nitrogen values were obtained by a micro-Kjeldahl procedure. The separation and the analyses of the acid-soluble, phospholipid and nucleic acid phosphorus fractions were done by the methods of Schneider (5).

RESULTS AND DISCUSSION

The data obtained from 29 patients are given in Tables I and II. With four exceptions, the concentration of the drug in the neoplasm was greater than in the corresponding normal tissue. The magnitude of the difference in concentration was quite variable. Values of from 1.5 to 2 times were commonly obtained and on 2 occasions the concentrations observed in breast carcinomas were more than 7 times those observed in the normal breast tissue of the same patient. Analyses of such other tissues as were available are included to emphasize the fact that distribution of this drug in the body is very uneven and that certain tissues (*e.g.*, spleen, lymph nodes and liver) characteristically contain even larger amounts than the neoplasms themselves.

There are doubtless several mechanisms at play in determining the degree of fixation of the drug in any given tissue, but it seems justifiable to conclude that there is some process occurring in neoplastic cells that increases this fixation in comparison with the "parent" tissue from which the neoplasm arose. The nature of this process is obscure. The ubiquitous role of phosphorus compounds in tissue metabolism suggested the analyses recorded in Table II. It can be seen that there is no obvious correlation between the quinine concentration and either the total nitrogen or the phosphorus values obtained on neoplastic and normal tissues.

That the process is not related to simple tissue damage or degeneration is shown by the lower concentrations observed in the involved areas of the mucosa and muscle of the patient with regional ileitis and in the cystic area of the breast in the patient with non-malignant diseased breast.

*This work was aided by grants from the Charles H. and Mary F. S. Worcester Memorial Fund, the O. C. Miller Memorial Cancer Research Fund and the Cinchona Products Institute.

SUMMARY

Analyses were made of the extent of quinine localization in a variety of human malignant neoplasms and corresponding normal tissues. In most cases, a higher concentration was observed in neoplastic tissue than in the tissue of origin. However, the concentration observed in liver, lymph nodes, spleen and certain other tissues was much higher than that observed in the neoplasms. No correlation was observed between the quinine concentration and the nitrogen or phosphorus content.

REFERENCES

1. KELSEY, F. E., and GEILING, E. M. K. Micro Determination of Quinine in Blood and Tissues. J. Pharmacol. & Exper. Therap., **75**:183-186. 1942.
2. KELSEY, F. E., OLDHAM, F. K., and GEILING, E. M. K. Studies on Antimalarial Drugs; Distribution of Quinine in the Tissues of the Fowl. J. Pharmacol. & Exper. Therap., **78**:314-319. 1943.
3. KELSEY, F. E., OLDHAM, F. K., and GEILING, E. M. K. Studies on Antimalarial Drugs; Metabolism of Quinine and Quinidine in Birds and Mammals. J. Pharmacol. & Exper. Therap., **85**:170-175. 1945.

TABLE I: CONCENTRATION OF QUININE IN NEOPLASTIC AND NON-NEOPLASTIC TISSUES AFTER ORAL INTRAVENOUS ADMINISTRATION OF QUININE

Patient No.	Diagnosis	Route*	Time after drug *Hr: Min.*	Concentration of quinine in plasma and tissues: Plasma** *mgm./l*	Carcinoma *mgm./kgm.*	Other tissues *mgm./kgm.*
25	Carcinoma, skin of orbit	I.V.	2:30	6.6	17.2	Skin, 12.7; muscle, 8.3
2	Carcinoma, tongue	Oral	4:10	3.5	15.6	Mucosa, 9.0; muscle, 9.0
28	Carcinoma, larynx	I.V.	2:30	3.5	5.2	Mucosa, 4.0; muscle, 3.8
39	Carcinoma, larynx	I.V.	3:00	3.5	10.5	Mucosa, 6.2; muscle, 4.8
1	Recurrent carcinoma, neck (Primary, larynx)	Oral	3:15	4.8	18.2	Skin, 11.8; muscle, 8.3; lymph nodes, 15.7; carotid artery, 7.6
18	Carcinoma, esophagus	I.V.	3:10	4.3	13.4	Esophageal mucosa, 10.0; gastric mucosa, 11.7; spleen, 46.3
33	Carcinoma, esophagus	I.V.	3:30	6.5	17.0	Mucosa, 12.0; muscle, 8.0; lymph nodes, 24.0
9	Carcinoma, stomach	I.V.	4:20	6.8	17.2	Gastric mucosa, 12.0; gastric muscle, 6.6; colon mucosa, 8.6; colon muscle, 6.7
27	Carcinoma, stomach	I.V.	2:00	5.0	19.0	Mucosa, 12.5; spleen, 24.0
29	Carcinoma, stomach	I.V.	3:00	8.7	15.3	Mucosa, 8.2; liver, 40.0; spleen, 18.0
37	Carcinoma, stomach	I.V.	4:00	7.0	12.3	Mucosa, 10.1
3	Carcinoma, colon	Oral	4:10	4.6	31.3	Mucosa, 15.5; muscle, 6.0; peritoneum, 2.2; anal skin, 14.3
13	Carcinoma, colon	I.V.	3:15	6.5	12.4	Colon mucosa, 7.9; ileal mucosa, 14.4; liver, 42.7
20	Carcinoma, colon	I.V.	3:30	4.6	30.5	Mucosa, 17.6; muscle, 6.7
30	Carcinoma, colon	I.V.	2:30	6.2	8.3	Mucosa, 12.5; muscle, 4.0; skin, 5.1
40	Carcinoma, colon	I.V.	7:40	4.6	5.3	Mucosa, 4.6; kidney, 8.3; spleen, 8.9
34	Carcinoma, anal colon	I.V.	3:45	4.5	5.3	Skin, 6.0
36	Carcinoma, rectum	I.V.	2:20	3.4	9.9	Mucosa, 6.2; muscle, 3.4
19	Carcinomatosis	I.V.	2:20	13.9	16.4	Abdominal wall muscle, 12.9; subcutaneous fat, 4.5
21	Carcinoma, skin	Oral	5:45	8.7	9.6	Normal skin, 14.0; cicatrized skin, 9.3; synovia from knee joint, 4.6; quadricepts tendon, 3.1
32	Carcinoma, cervix	I.V.	3:30	7.2	48.5	Mucosa, 21.0; vaginal epithelium, 10.0
8	Carcinoma, breast	Oral	3:20	7.5	38.0	Pectoral muscle, 9.0; normal breast, 4.8; breast fat, 3.0; skin, 5.9; lymph nodes (involved), 16.3
23	Carcinoma, breast	Oral	4:50	5.4	10.9	Pectoral muscle, 8.5; normal breast, 8.0; skin, 6.8; lymph nodes (involved), 24.0
35	Carcinoma, breast	I.V.	3:15	2.7	11.0	Pectoral muscle, 4.5; normal breast, 1.5; skin, 2.5; lymph nodes (involved), 8.0
14	Carcinoma, head of pancreas	I.V.	2:45	2.3	10.3	Normal pancreas, 10.8; gastric mucosa, 6.9; duodenal mucosa, 5.1
22	Duodenal ulcer	I.V.	1:45	3.8	..	Pyloric mucosa, 13.2; fundus mucosa, 14.5; muscle, 6.1
7	Diseased breast (non-malignant)	Oral	3:00	3.9	..	Normal breast, 2.3; cystic breast, 1.9
5	Inflammatory "tumor," thigh	Oral	4:10	7.1	..	"Tumor," 9.4; muscle, 5.6; skin, 8.0; subcutaneous fat, 2.4
6	Regional ileitis	Oral	2:15	7.2	..	Uninvolved mucosa, 18.8; involved mucosa, 13.6; uninvolved muscle, 9.9; involved muscle, 6.6

*The dosage used was 1.0 gm. by mouth or 0.5 gm. by vein.

**Calculated from data obtained on blood drawn before and after tissues were removed.

4. Kelsey, F. E., and Brunschwig, A. Concentration of Quinine in Gastrointestinal Cancers. Preliminary Report. J. Nat. Cancer Inst. **7**:355-356. 1947

5. Schneider, W. C. Phosphorus Compounds in Animal Tissues. I. Extraction and Estimation of Desoxypentose Nucleic Acid and of Pentose Nucleic Acid. J. Biol. Chem., **161**:293-303. 1945.

Table II: Nitrogen and Phosphorus Analyses of Neoplastic and Non-Neoplastic tissues

Patient no.*	Site of carcinoma	Tissue	Quinine as % of plasma concentration	Total nitrogen (%)	Phospholipid phosphorus (*mg%*)	Nucleic acid phosphorus (*mg%*)	Acid soluble phosphorus (*mg%*)
28	Larynx	Carcinoma	150	2.5	45	45	43
		Mucosa	110	2.0	26	25	22
		Muscle	110	3.0	31	11	87
33	Esophagus	Carcinoma	260	2.9			
		Mucosa	180	2.5			
		Muscle	120	2.9			
		Lymph node	370	3.0			
27	Stomach	Carcinoma	380	2.2	56	59	
		Mucosa	250	1.6	52	64	
		Spleen	480	3.4	73	105	
29	Stomach	Carcinoma	180	2.6	70	90	88
		Mucosa	90	2.6	36	53	77
		Liver	460	3.0	83	61	101
		Spleen	210	2.9	72	86	100
37	Stomach	Carcinoma	180	2.4			
		Mucosa	140	2.3			
13	Colon	Carcinoma	190	2.0			
		Liver	660	1.9			
30	Colon	Carcinoma	130	1.9	33	78	60
		Muscle	70	1.8	52	12	63
32	Cervix	Carcinoma	670	2.5			
		Mucosa	290	1.8			
		Vaginal epithelium	140	2.0			
8	Breast	Carcinoma	510	3.2			
		Pectoral muscle	120	2.2			
35	Breast	Carcinoma	410	3.1	31	84	114
		Pectoral muscle	170	3.5	47	12	167
		Lymph nodes (involved)	300	1.3	45	48	71

*For further details of the results obtained on each patient, *see* Table I.

Observations on the Ketosteroid Content of Urine from Patients with Prostatic Carcinoma and Adenoma*

E. W. McHenry, M. A., Ph. D., F. R. S. C., E. M. Semmons, M. A., R. Pearse, F. R. C. S., and E. G. Meyer, M. D., F. R. C. S. (C).

(*From the Connaught Medical Research Laboratories and Department of Surgery, University of Toronto, Toronto, Canada*)

(Received for publication February 17, 1947)

The relation of sex hormones to enlargement of the prostate was reviewed by Dodds (2). Observations by Huggins and associates (5) led to the conclusion that androgens stimulate activity of the prostate and that estrogens have a depressing effect. Dean (1) reported observations on the androgen content of urine from cases of prostatic carcinoma before and after treatment by castration and by administration of estrogens. Urinary levels prior to castration appeared to be low. Data on urinary excretion of androgens have been reported by Fraser and associates (3), mainly for normal subjects and for persons with endocrine disorders. The content of 17-ketosteroids in the urine of patients with prostatic cancer was determined before and after castration by Scott and Vermeulen (6). Normal values have been given also by Venning and Kazmin (7). Since there has been a wide-spread assumption that sex hormones were involved in the production of carcinoma of the prostate, it seemed advantageous to determine urinary excretions of ketosteroids by patients having carcinomas or adenomas of the prostate.

METHODS

The quantity of 17-ketosteroids was determined chemically by a modification of the procedure of Holtorff and Koch (4), involving an adaptation of the Zimmerman color reaction (8). Briefly, the method involves hydrolysis to liberate the ketosteroids from esters, extraction of the ketosteroids with benzene, removal of phenolic material with sodium hydroxide, and the development of color by combination of ketosteroids with *m*-dinitro benzene in the presence of aqueous potassium hydroxide. Color determinations were made in the Coleman universal spectrophotometer set at 520 mμ., using pure androsterone as a standard. Neutral 17-ketosteroids were calculated as androsterone. Recovery of known amounts of androsterone added to urine samples averaged 94 per cent.

Except in special cases noted below, 24-hour specimens of urine were used. Usual preservatives, such as toluene and chloroform, which are solvents for ketosteroids, are unsatisfactory for this analysis. Benzoic acid was found to be suitable since it preserved the urine and did not interfere with the determination. It was employed in a concentration of 2 gm. per collection bottle (0.1 to 0.2 per cent weight by volume). Urine specimens were kept cool whenever possible, and analyses were made shortly after collection was complete.

Under prevailing conditions of staff shortages in hospitals, there was some difficulty in collecting 24-hour specimens. It was suggested that morning specimens would give sufficient information. Analyses of samples from 2 normal subjects, obtained thrice daily for 4 consecutive days, showed considerable variation in 17-ketosteroid content. Contrary to expectation, the morning specimen did not always have the highest concentration. Because of the variability found in these specimens, it seemed advantageous to use only 24-hour samples.

Observations on cases of prostatic carcinoma and adenoma.—About 200 specimens were obtained from 32 cases of carcinoma and from 39 cases of adenoma. The levels were contrasted with those found in specimens from 19 normal males. Data are given in Table I. The range of values found for normal subjects is in agreement with the observations for similar persons reported by Fraser and associates (4), by Scott and Vermeulen (6) and by Venning and Kazmin (7). It should be noted that normal subjects were appreciably younger than persons in the carcinoma and adenoma groups. It was not possible to obtain specimens from normal men of older ages. Total 24-hour excretion of ketosteroids by cases of carcinoma and adenoma of the prostate were fairly similar and were definitely lower than values found for younger normal persons. If prostatic carcinoma results from, or is coincident with, an abnormal secretion of androgenic hormones, an increased output in the urine would be expected.

*This investigation was aided by a grant from the Ontario Cancer Treatment and Research Foundation.

This was not the case in the patients on whom observations were made. The lower values actually found were similar to the precastration data reported by Dean (1) and are probably a reflection of the ages of the subjects. They are intermediate between the values for older subjects found by Fraser and associates (3) and by Venning and Kazmin (7).

TABLE I: 24-HOUR EXCRETION OF 17-KETOSTEROIDS

	A Controls	B Adenoma of prostate	C Carcinoma of prostate
Number of subjects	19	39	32
Age in years	20–60	48–82	56–89
Range	10.1–23.2 mgm.	2.8–18.2 mgm.	2.7–16.8 mgm.
Average	16.2 mgm.	10.2 mgm.	10.0 mgm.
Standard deviation	4.02 mgm.	4.00 mgm.	4.12 mgm.
Correlation (r) with urine volume	0.4930*	0.1370	0.4732†

*Significant at the 5 per cent level.
†Significant at the 1 per cent level.

Relation of ketosteroid output and urine volume.—In considering the data shown in Table I it was observed that there was some indication of a relation of the total amount of ketosteroids to the volume of urine. Calculation of correlation coefficients showed that the relation was significant at the 5 per cent level for normal subjects and at the 1 per cent level for carcinoma patients. The relation was not significant for adenoma patients.

To obtain further information on a correlation between volume of urine and output of ketosteroids, 24-hour specimens were obtained, for 13 consecutive days, from a normal male, 45 to 50 years of age. During the first 9 days no attention was paid to ingestion of fluids and the variation in output was small. The correlation coefficient for this period was very slightly less than significant at the 5 per cent level. During the remaining 4 days, the subject took large amounts of fluids. Urinary volume and content of 17-ketosteroids were markedly increased, the latter by about 50 per cent. For this entire series of specimens, the correlation between volume and total output of 17-ketosteroids was significant at the 1 per cent level. The data are given in Table II.

Relation of ketosteroid concentration to specific gravity.—Arising from the observations on the relation between volume and the total daily excretion of ketosteroids, it seemed advantageous to determine if there was also a relation between specific gravity of urine and the concentration of ketosteroids (*i.e.*, the amount per unit of volume).

TABLE II: 17-KETOSTEROID EXCRETION OF A NORMAL MALE, 45-50 YEARS OF AGE
RELATION OF URINE VOLUME TO TOTAL EXCRETION, (mgm./24 hours)

	9 consecutive days	13 consecutive days
Range	14.6–18.7	14.6–24.0
Average	16.3	16.8
Standard deviation	1.70	2.66
Correlation (r)	0.5369	0.7669†

†Significant at the 1 per cent level.

For this series of samples from a normal subject referred to in the previous section, there was a highly significant correlation. The data are given in Table III. This correlation was not suspected until after analyses had been made on a considerable number of the hospital specimens and for these earlier samples specific gravity was not measured. Hospital specimens on which specific gravity was determined showed an indication of the same correlation, as determined by plotting specific gravity against ketosteroid concentration.

TABLE III: 17-KETOSTEROID EXCRETION OF A NORMAL MALE, 45-50 YEARS OF AGE RELATION OF SPECIFIC GRAVITY OF URINE TO CONCENTRATION, (mgm./100 cc.)

	9 consecutive days	13 consecutive days
Range	1.18–1.84	0.88–1.84
Average	1.48	1.34
Standard deviation	0.26	0.29
Correlation (r)	0.8723†	0.9012†

†Significant at the 1 per cent level.

DISCUSSION

The data given in Table I show that in a series of 32 patients, in whom a diagnosis of prostate carcinoma was definitely made, there was not an abnormally high output of 17-ketosteroids in the urine. In fact the output was less than that observed in normal males of younger age. The amounts observed were probably compatible with the ages of the patients. The data are in agreement with the report of Scott and Vermeulen (6). It is concluded that the estimation of 17-ketosteroids in the urine is of no value as a guide to hormone therapy in the treatment of carcinoma of the prostate.

The observed relations between daily output of ketosteroids and urinary volume and between urine specific gravity and ketosteroid concentration cause doubt to arise as to the value of determinations of ketosteroids in urine for diagnostic purposes. The assumption is made frequently that estimation of a constituent of urine is an indication of the amount of the constituent in the blood and in the body. The data here recorded give an impression that the daily excretion of ketosteroids varies with the amount of urine

and that the concentration of ketosteroids parallels the amount of solids in the urine. These data indicate that, in some cases at least, the excretion of ketosteroids is dependent upon kidney function and is not a reflection of the amount of ketosteroids available in the body. Some indications are available, however, which lead us to think that the two relations described above hold only when the metabolism of 17-ketosteroids is approximately normal. Specimens from young children and from cases of adrenal tumor and of pituitary tumor have not shown these relations. These observations suggest an approach to the interpretation of analyses of ketosteroids in urine. If values for a series of normal specimens are available, a curve showing either the relation of total output to urine volume or of concentration to specific gravity can be plotted. An observation failing to fit the curve, within the limits of error, may be regarded as abnormal. On this basis the values for carcinoma patients shown in Table I may be considered as "normal." In our opinion data regarding ketosteroids in urine should be considered in conjunction with urinary volume or specific gravity, depending upon whether total excretion or concentration is measured. Unless this is done, misleading conclusions may be drawn.

SUMMARY

Colorimetric estimations of 17-ketosteroids in urine specimens from cases of prostatic carcinoma and adenoma gave values for daily output less than those found for normal males of younger age. The low values are probably normal for the ages of the patients. In these patients the estimation of urinary ketosteroids was of no value in the diagnosis of carcinoma of the prostate and was not useful as a guide to hormone therapy. For the normal controls and for the carcinoma patients there were correlations between the daily output of ketosteroids and the volume of urine, and between the concentration of ketosteroids in the urine and its specific gravity. It is suggested that these correlations, indicative of the effect of kidney function, should be kept in mind in estimations of ketosteroids in urine.

REFERENCES

1. Dean, A. L., Woodward, Helen Q., and Twombly, G. H. The Endocrine Treatment of Cancers of the Prostate. Surgery, **16**:169-180. 1944.
2. Dodds, E. C. Hormones in Cancer. Vitamins & Hormones, **2**:353-359. 1944.
3. Fraser, R. W., Forbes, Anne P., Albright, F., Sulkowitch, H., and Reifenstein, E. C. Colorimetric Assay of 17-Ketosteroids in Urine. Survey of the Use of this Test in Endrocrine Investigation, Diagnosis and Therapy. J. Clin. Endrocrinol., **1**: 234-256. 1941.
4. Holtorff, A. F., and Koch, F. C. The Colorimetric Estimation of 17-Ketosteroids and Their Application to Urine Extracts. J. Biol. Chem., **135**:377-392. 1940.
5. Huggins, C., and Hodges, C. V. Studies on Prostatic Cancer. I. The Effect of Castration, of Estrogen and of Androgen Injection on Serum Phosphatases in Metastatic Carcinoma of the Prostate. Cancer Research, **1**:293-297. 1941.
6. Scott, W. W., and Vermeulen, C. Studies on Prostatic Cancer. V. Excretion of 17-Ketosteroids, Estrogens and Gonadotropins before and after Castration. J. Clin. Endocrinol., **2**:450-456. 1942.
7. Venning, Eleanor H., and Kazmin, V. Excretion of Urinary Corticoids and 17-Ketosteroids in the Normal Individual. Endocrinology, **39**:131-139. 1946.
8. Zimmermann, W. Eine Farbreaktion der Sexualhormone und ihre Anwendung zur Quantitativen Colorimetrischen Bestimmung. Ztschr. f. physiol. Chem., **233**:257-264. 1935.

Cancer Mortality In Egypt

Mahmoud Ahmed Afifi,* M. B., CH. B (Cairo), M. R. C. S. (England)
L. R. C. P. (London), D. M. R. E. (Cambridge)

(Received for publication January 19, 1947)

One aspect of the study of cancer is the examination of records of the disease as it manifests itself in various parts of the world. The mortality records of the separate countries are fertile sources of information concerning the effects on the disease of varied climatic conditions, soils, diets, social habits, and race. Although death reports of the European countries as well as those of the United States have been extensively tabulated and studied, examination of reports for countries such as Egypt have not often been made. The reported cancer mortality rate in Egypt was 27.2 per 100,000 inhabitants in 1942. This rate is low compared with 122.1 in the United States and 183.4 in England and Wales for the same year. Its meaning with respect to the incidence of cancer in Egypt merits consideration, and forms the objective of the present paper.

Population Characteristics of Egypt

The population of Egypt in 1937 was 15,920,000; estimates for 1942 give the number as 17,230,000. The population is densely concentrated (over 1,000 per square mile) along the Nile Valley and in the oases, the inhabitable portion of Egypt comprising an area roughly equivalent to Holland. One-fourth of the population resides in urban towns and three-fourths in the rural area although in the latter it is practically impossible at any time to be out of sight of human beings except by going under cover. The rural population predominance is also shown by data on occupation: 58 per cent of the occupations specified in the 1937 census relate to agriculture and only 6 per cent to industry.

The age distribution is in favor of the younger ages, nearly half (48 per cent) being under 20 years of age, as contrasted to 30 per cent in England in 1942, and 34 per cent in the United States in 1940, while the percentage over 60 years of age was 6.4 compared with 15.8 in England, 1942, and 10.4 in the United States in 1940.

According to the census of 1937 the foreign-born population of Egypt included 187,000 persons, or 1.2 per cent of the total population. In the governorates and capitals of provinces they number 177,000 or 94.6 per cent of the total foreign-born population. In Alexandria and Cairo foreigners comprise 12.8 and 4.6 per cent of the population, respectively. The foriegn-born are thus largely concentrated in the large urban centers.

Mortality Registration

The death registration system does not cover all of Egypt. Before 1925 registration was limited to the thirty governorates and capitals of provinces and comprised a total population of 2,000,000. In 1926 the registration area was extended to include all places where public health departments existed, a population of about 3,000,000. The area newly included consisted for the most part of small towns or large villages, an essentially rural population. Several years were necessary before the newly formed registration bureaus began to function properly and the year 1933 has been taken as the beginning of the present stabilized registration area.

The registration area in 1942 contained 5,590,000 persons or 32.4 per cent of the total population. About half of these were in Cario and Alexandria, and a large proportion of the other half was also to be classed as urban although data unfortunately are not available to permit a more exact description of the urban-rural distribution of the area.

This point is important in evaluating the completeness and accuracy of death reporting in Egypt. In rural districts outside the registration area deaths are not recorded as to cause beyond the terms "accidental" or "non-accidental", and a gross deficiency in recording the cause of death must also prevail in the rural sections most recently admitted to the registration area, particularly since about half the death certificates filed in Cairo and Alexandria do not show the cause of death. If this is true in the most advanced and medically best equipped section of the registration area, very little can be said for the poorer rural sections.

The failure to secure proper certification of cause in more than 50 per cent of the deaths in the two

* Former Director of the Radiological and Electro-Therapeutic Departments of the Government Hospital in Alexandria. Present address: Roentgen Institute, 38 Saad Zagloul Street, Alexandria, Egypt.

large cities may be laid to the general level of ignorance of the people. Beset with superstition and a belief in the efficacy of the herbs and the medications prescribed by the quacks and laymen they patronize, many die before proper medical attention can reach them. The cause of death in such cases remains unknown unless special investigation is made by medical officers of health. Such investigations are made and often do disclose the real cause of death, but many cases remain in which the true cause is never known. The picture becomes still more confused when one considers the fact that no proper control over certification is maintained so that many deaths from cancer, for example, may have been certified as due to some other cause.

The remarks contained in the paragraphs to follow must be considered in the light of the characteristics of the system of mortality reporting just described: a registration area comprising less than one-third the total population and predominantly urban in an essentially rural country, a deficiency of 50 per cent in medical certification of death in the better sections of the area, and no control over the diagnostic certification of death.

Cancer Mortality—General

The recorded death rate per 100,000 from cancer varied between 17.7 in 1927 and 27.6 in 1935 over the period 1918-1942. The general level of mortality may be contrasted with that for other countries in Table I, for the years 1926-1928 (12).

Table I: Cancer Death Rates Per 100,00 Population for Various Countries, 1926-1928

Country	1926	1927	1928
Australia	93.3	92.4	94.8
Belgium	84.7	86.8	91.0
Bulgaria	——	63.0	——
Czechoslovakia	107.5	107.9	110.8
Denmark	140.0	141.0	144.0
Egypt	20.5	17.7	19.6
England and Wales	136.2	137.6	142.5
Finland	64.4	79.2	84.1
France	87.7	95.0	97.0
Germany	118.0	123.0	126.0
Greece	29.0	34.0	32.2
Japan	68.0	70.0	——
Lithuania	——	31.0	31.4
Mexico	17.0	15.7	——
New Zealand	99.1	96.3	98.8
Spain	——	68.0	70.1
Sweden	120.2	119.8	——
Switzerland	143.0	150.0	145.0
United States	94.9	95.6	96.1

With the exception of the rates for Mexico, Egypt's cancer mortality is lowest on the list. Rates for Czechoslovakia, Denmark, England, Germany, Sweden, Switzerland, and the United States are from 5 to 7 times the rate for Egypt.

The trends of cancer mortality in the registration area of Egypt, and in the two principal cities, are shown in Fig. 1. The period prior to 1926 was one of a constant rate varying between 22 and 26. This was the period during which the registration area was limited to the 2,000,000 population of the governorates and capitals of provinces. The rate fell during the years 1926-1932, following the extension of the registration area to cover another

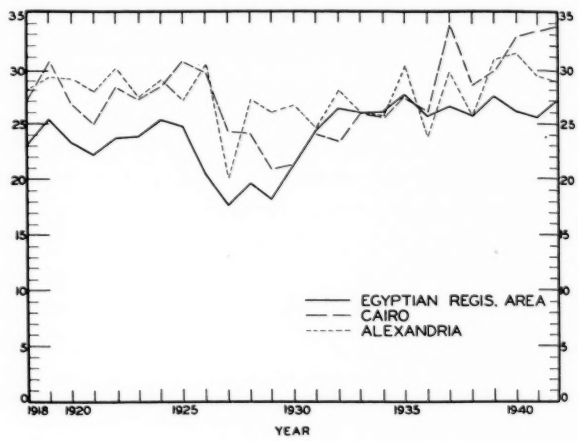


Fig. 1.—Trend of cancer mortality in Egyptian Registration Area, in Cairo and in Alexandria. Deaths per 100,000 population.

1,000,000 in the rural regions, and slowly recovered as registration gradually improved. Following 1932 the rate for the registration area has remained constant at a level slightly above that for the first period. In the two cities, however, the rate has shown a fairly steady rise, particularly in Cairo.

A regional presentation of cancer mortality is given in Fig. 2. The death rates are for the year 1942, and for the cities and towns designated. Rates for the rural sections cannot be computed because of the lack of rural population data for the registration area. The rates range from 42.6 in the governorate of Ismailia to 8.9 in that of Damietta and 4.5 in the chief town of Asswan. The wide range of rates may be ascribed in part to the relatively small population in many of the towns and governorates. The figure shows no special concentration of either high or low rates in any particular region.

Data from Biopsies and Autopsies

Out of 20,280 specimens from biopsies and autopsies sent for examination to the pathological laboratory of the Ministry of Public Health, Cairo, from 1928 to 1937 inclusive, 3,031 or 15 per cent

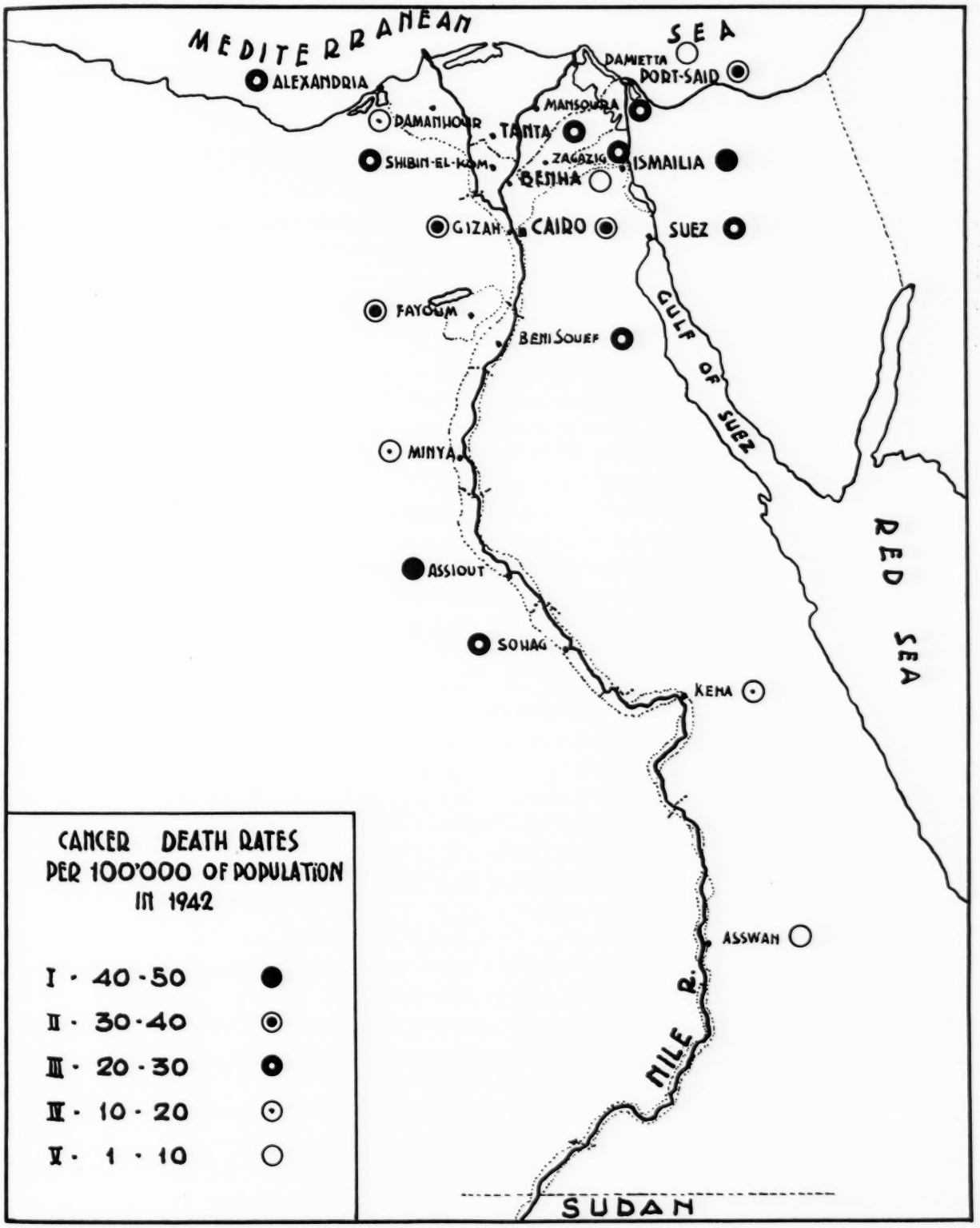


FIG. 2.—Geographical distribution of cancer mortality in Egypt, 1942.

were classified as benign and 3,180, or 15 per cent, as malignant. The remaining specimens were not tumors (7).

Of 651 specimens taken from hospital patients of the Government Hospital in Alexandria from September, 1931 to November, 1938, 330 cases (15 per cent) were classified as benign and 401 (18 per cent) as malignant. The malignant lesions were classified as carcinomas, 292 (72 per cent); sarcomas, 78 (19 per cent); endotheliomas, 9 (2 per cent); and other tumors, 22 (6 per cent) (8).

During the period 1931-1938, 880 autopsies were made in the Alexandria Government Hospital; 79 of these (9 per cent) showed malignant disease (8). In Kasr-el-Ainy Hospital in Cairo, 5,924 postmortem examinations were made during the years 1905-1930 inclusive. Malignancy was found in 365 cases, or 6.2 per cent. The cases of malignant disease were analyzed by Professor M. F. Sorour (16) and were as listed in Table II.

TABLE II: SOROUR'S ANALYSIS OF 5,924 POSTMORTEM EXAMINATIONS MADE IN PERIOD OF 1905-1930 INCLUSIVE

Site of malignant disease	Number of cases			Percentage of total
	Epithelioma	Sarcoma	Total	
Lip	10	—	10	2.7
Tongue	9	—	9	2.5
Larynx	8	—	8	2.2
Esophagus	20	—	20	5.5
Stomach	13	—	13	3.6
Liver	15	—	15	4.1
Gall bladder	10	1	11	3.0
Pancreas	11	—	11	3.0
Colon	13	—	13	3.6
Rectum	11	—	11	3.0
Bladder	80	5	85	23.3
Cervix uteri	12	—	12	3.3
Corpus uteri	9	—	9	2.5
Ovaries	8	—	8	2.2
Breast	15	—	15	4.1
Kidney	5	4	9	2.5
Retroperitoneal	—	27	27	7.4
Mediastinum and lung	2	13	15	4.1
Brain and dura	—	6	6	1.6
Thyroid gland	3	1	4	1.1
Other parts of the body	—	54	54	14.8
Total	254	111	365	100.0

CANCER MORTALITY BY SITE

Tabulations of cancer deaths by anatomical site and by sex are available for the years 1931-1942, inclusive, and are summarized in Table III. The male genital organs comprised the most frequent location of fatal cancer with 1,451 deaths (aside from the catch-all group of "other and unspecified" organs). Deaths from malignant disease of the stomach and duodenum accounted for the next largest number, 1,337. These deaths were approximately evenly divided between males and females. Disease involving the breast and uterus each accounted for somewhat over 1,200 deaths, while cancer of the liver and biliary passages, and of the respiratory tract, follow in order. Over twice as many deaths from cancer of the respiratory tract were found for males than for females, which was true also of cancer of the buccal cavity and pharynx. More deaths from malignant disease in other and unspecified organs were recorded for females than males, possibly because of a less precise diagnosis of cancer in the Mohammedan women of Egypt.

The trend of cancer mortality by site over the period 1931-1942, inclusive, is shown in Fig. 3. Rates for cancer classified as "other organs of the digestive tract" (Table III), of the male genito-urinary system, and of the respiratory system have shown a marked increase since 1936. A somewhat erratic but definite increase in mortality from cancer in other and unspecified organs is also shown. Mortality from malignancy of the esophagus, stomach and rectum, and of the breast shows a slight decrease, but not a significant one. Mortality from cancer of the skin decreased from a level of 0.3 per 100,000 during 1931-1935 to a rate of 0.1 in the years 1936-1942. No trend is evident in the death rates from cancer of the liver and biliary passages, of the uterus, and of the buccal cavity.

Some indication is given in these data of an increase in the mortality of cancer of the deep-seated organs (*i.e.* of the intestines and the male genito-urinary tract) and a decrease or leveling-off of deaths from cancer of the more accessible parts of the body (skin, uterus, and buccal cavity). If these facts can be interpreted in the same manner as similar findings in the United States, they may be cited as evidence of an improvement in diagnosis. The anti-bilharzial hospitals, stationary and mobile, increasing in number during the period, and examining large groups of the population, must have helped to reveal and record cases of cancer of the more deep-seated organs which otherwise would have been easily missed.

The distribution of Egyptian cancer deaths by site is compared with similar distribution for England and Wales and for the United States in Table IV. Although roughly comparable, it is seen that the site most frequently recorded, digestive organs and peritoneum, comprise only 34.4 per cent of the total as against 52.7 and 45.9 per cent in England and Wales, and in the United States, respectively. Deaths comprising the somewhat anomalous grouping, "male genital organs and urinary

TABLE III: CANCER DEATHS AND DEATH RATES BY SITE AND BY SEX FOR THE 12-YEAR PERIOD, 1931-1942. EGYPTIAN REGISTRATION AREA

SITE	Deaths			Annual rate/100,000		
	Male	Female	Total	Male	Female	Total
Buccal cavity and pharynx	388	164	552	1.4	0.5	1.0
Digestive tract and peritoneum						
Esophagus	67	29	96	0.2	0.1	0.2
Stomach and duodenum	683	654	1337	2.4	2.3	2.4
Rectum	296	144	440	1.0	0.5	0.8
Liver and biliary passages	847	836	1683	3.0	2.9	2.9
Pancreas	77	40	117	0.3	0.1	0.2
Peritoneum	43	50	93	0.1	0.2	0.2
Other organs, including intestine	525	372	897	1.8	1.3	1.6
Respiratory tract	747	269	1016	2.8	1.0	1.9
Uterus	——	1216	1216	—	4.3	2.1
Other female genital organs	——	98	98	—	0.4	0.2
Breast	48	1181	1229	0.1	4.1	2.1
Male genital organs	1451	——	1451	5.0	—	2.5
Skin	52	46	98	0.2	0.2	0.2
Other nonspecific organs	1078	1205	2283	3.8	4.2	4.0
Total	6302	6304	12606	22.0	21.9	22.1

organs of both sexes," form nearly twice as high a proportion of cancer deaths as in the other two countries. The greater difference, however, is to be found for the catch-all grouping of "other and nonspecified organs," which bulks greatly over the similar figures for the other countries. If this rubric were reduced to comparable size through more accurate reporting and better diagnosis, it is probable that the distribution of cancer deaths by site in Egypt would conform very closely with those for England and Wales, and the United States.

CANCER MORTALITY BY AGE AND SEX

The distribution of cancer deaths by age and sex is available for the registration area of Egypt for the year 1942 (Table V).

Deaths are fairly evenly proportioned between the sexes for each age group although an excess is shown for the males for ages 10 to 24 and 45 to 54, and for the females for ages 55 to 64 and 75 years and over. The death rates by age groups increase over the life span from 1.4 for ages under 10 years to 200.0 for ages 75 years and over. Contrasted with similar rates for the United States and for England and Wales for comparable years, rates for Egypt are the lowest for each age group. The difference, however, is less at the younger ages than at the older ones; at ages under 55 years the rates for Egypt are about half those for the other two countries. Above the age of 55, however, the disparity increases until the rate for 75 years and over is only about one-sixth of the corresponding rate for the other two countries.

TABLE IV: PERCENTAGE DISTRIBUTION OF CANCER DEATHS BY ANATOMICAL SITE, EGYPTIAN REGISTRATION AREA, 1931-1942, ENGLAND AND WALES, 1938-1944, AND THE UNITED STATES, 1943

Anatomical site	Egyptian Regis. Area 1931-1942			England and Wales* 1938-1944			United States† 1943		
	Males	Females	Total	Males	Females	Total	Males	Females	Total
Buccal cavity and pharynx	6.2	2.8	4.5	7.0	1.5	4.1	5.4	1.3	3.2
Digestive organs and peritoneum	40.2	28.0	34.4	57.8	47.9	52.7	52.3	40.1	45.9
Respiratory system	11.8	4.6	8.4	16.0	4.1	9.9	11.3	3.1	7.0
Uterus	——	20.9	10.0	——	12.4	6.4	——	19.4	10.1
Other female genital organs‡	——	1.7	0.8	——	6.5	3.4	——	5.3	2.8
Breast	0.8	20.5	10.2	0.2	19.8	10.3	0.3	18.2	9.7
Male genital organs	23.0	——	12.0	7.5	——	3.7	12.3	——	5.8
Urinary organs				4.7	2.4	3.5	6.2	3.2	4.8
Skin (scrotum excepted)	0.8	0.8	0.8	1.7	1.3	1.5	2.5	1.5	2.0
Brain and other parts of nervous system	17.2	20.7	18.9	1.3	0.9	1.0	2.0	1.1	1.5
Other or unspecified organs				3.8	3.2	3.5	7.7	6.8	7.2
Total, all sites	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

* These data were computed from data kindly supplied to me by the Rt. Hon. Lord Amulree, Ministry of Health, Whitehall, London.
† These data were computed from data kindly supplied to me by Mr. J. C. Capt, Director of the Bureau of the Census, Department of Commerce, Washington, D. C.
‡ The data, for Egypt, include female urinary organs.

TABLE V: DEATHS IN THE EGYPTIAN REGISTRATION AREA BY AGE AND SEX, 1942; WITH AGE SPECIFIC DEATH RATES COMPARED WITH THOSE OF THE UNITED STATES, 1940, AND ENGLAND AND WALES, 1942

Age	Deaths, Egypt Regis. Area, 1942				United States 1940	England and Wales 1942
	Male	Female	Total Number	Rate*	Rate*	Rate*
0– 9	10	8	18	1.4	3.8	3.5
10–24	29	16	45	3.3	4.6	4.5
25–34	34	39	73	10.2	17.3	18.1
35–44	95	93	188	31.1	61.1	63.6
45–54	154	137	291	76.2	168.8	186.1
55–64	156	174	330	142.2	369.6	419.9
65–74	113	103	216	171.4	695.2	824.7
75 and over	68	86	154	200.0	1183.9	1162.7
All ages	659	656	1315	27.2	120.3	183.4

* Per 100,000 population

NOTE: The rates for this table were computed by the use of population estimates for each group secured by apportioning the estimated population for the registration area according to the age distribution of the 1937 census of Egypt. Accuracy of the estimates depends upon the agreement between the age distribution of Egypt and that of the registration area for the country. Data necessary for this comparison are not available.

It was previously pointed out that the age distribution of the population of Egypt differs from that of the United States or of England and Wales in having a greater percentage of younger persons and a smaller percentage of older persons. The difference thus operates in the direction of a smaller total cancer death rate for all ages. The extent of the age effect can be shown. If the age distribution of Egypt had been the same as that for the United States in 1940, and the cancer death rates for Egypt shown in Table V were operative, the mortality rate from cancer for all ages would have been 39.8 instead of 27.2. The increase of 12.6, or 46 per cent, although large, clearly shows that the factor of age does not account for the relatively small total cancer death rate recorded for Egypt.

CANCER MORTALITY COMPARED WITH THAT OF OTHER DISEASES

In view of the low cancer mortality rate in Egypt compared with that of other countries it is interesting to note that an entirely different situation exists with regard to mortality from all causes of death. We have available for study the total recorded deaths in the registration area of Egypt for the period 1918-1942 and a tabulation by cause of death for the ten year period 1932-1941. The average annual mortality rate for all causes was 32.2 per 1,000 population for the period 1918-1942; for the period 1932-1941 in the expanding registration area, the rate was 36.8. Similar rates for the United States and for England and Wales in 1940 were 10.8 and 13.8 respectively (exclusive of deaths of civilians due to operations of war in England and Wales). The death rate for all causes in Egypt is thus approximately three times as great as that for the United States, and for England and Wales, in spite of a presumed under-registration of deaths and a very favorable age distribution.

Death rates for the principal causes of death are shown in Table VI. Diarrhea and enteritis, the respiratory diseases, tuberculosis, the other infections and parasitic diseases, heart disease, and the nonvenereal urinary and genital diseases all rank higher in the list than cancer. The *prima facie* evidence of this table points to a low prevalence of cancer in Egypt.

However, other facts lead us to accept this evidence with great caution. Cancer outranks nearly all the other diseases on the list in difficulty of diagnosis. The excessive rates for the rubrics "senility" and "other causes" indicate that, in general, diagnostic certification of deaths is poor in Egypt. These two groups together form 28 per cent of all deaths in Egypt in contrast to 13 and 10 per cent in the United States and in England and Wales, respectively. It is a well-known fact that in regions where medical service and death certification are poor, assignments of cause of death to senility and to "ill-defined causes" (included in Table VI in the "other cause" rubric), are high. It will also be recalled from the preceeding paragraphs, that criticism of death registration in Egypt is in general directed towards certification of cause of death, and not towards the actual enumeration of numbers of deaths.

Further, it is easy to establish the fact that the rate of cancer mortality is markedly low in areas of defective death registration. As an illustration, tables of mortality were examined for 52 counties in 10 states in the United States in which registration was notably poor in 1920. The cancer death rate per 100,000 was 12.5 in this group of counties, half that of the rate in Egypt (23.3) in the same year, and but 15 per cent of the cancer death rate in the American death registration area in 1920. The point seems clear that a low level of cancer mortality can be accepted only after abundant demonstration that the mechanism of death certification and reporting is well set up and functioning properly.

It is interesting, before closing the present section, to compare the various causes of mortality in Egypt with those of the United States, and of England and Wales, as shown in Table VI. The outstanding contrast is for diarrhea and enteritis; mortality from this cause is 10 times greater in Egypt than in the other two countries. Excessive mortality is also indicated for the respiratory

TABLE VI: DEATH RATES FROM PRINCIPAL CAUSES OF DEATH IN THE EGYPTIAN REGISTRATION AREA 1932-1941, THE UNITED STATES, 1940, AND IN ENGLAND AND WALES, 1940

Cause of death	Annual rates/100,000 population		
	Egypt 1932-1941	United States 1940	England and Wales 1940
Tuberculosis	66.1	45.9	69.9
Syphilis	10.2	14.4	8.0
Malaria	1.3	1.1	0.1
Dysentery	10.9	1.9	0.5
Other infections and parasitic diseases	124.9	27.7	52.0
Cancer and other malignant tumors	26.9	120.3	172.3
Nonmalignant or unspecified tumors	1.7	5.0	5.7
Diabetes mellitus	19.4	26.6	13.4
Pneumonia	307.9	54.9	72.7
Bronchitis	258.6	3.0	115.9
Other respiratory diseases	64.5	8.2	21.5
Cerebral hemorrhage	24.5	88.2	124.2
Hemiplegia and other paralyses of unstated origin	38.3	2.7	5.2
Heart disease	124.3	292.5	341.6
Other circulatory disease	17.0	21.9	39.4
Disease of the liver and biliary passages	17.8	15.8	6.0
Urinary and genital diseases, not venereal	135.0	95.4	60.4
Puerperal mortality	16.1	6.7	4.1
Diarrhea and enteritis	1255.2	10.3	11.1
Senility	347.7	7.7	48.2
Violence	124.4	94.3	118.3
Other causes	683.3	131.9	89.4*
All causes	3675.7	1076.4	1379.9*

* Exclusive of deaths of civilians due to operations of war.

diseases although this finding is hardly established by the data of Table VI, since the rates for the other two countries are only for 1 year. However, during the 9 year period 1932-1940 the rates for respiratory disease varied between 66 and 105 in the United States, and between 145 and 210 in England and Wales so that the respiratory disease rate of 631 for Egypt is approximately 6 times that for the United States and 3 times that for England and Wales.

Deaths from chronic disease are low in Egypt. The rates for cerebral hemorrhage, and circulatory disease, are lower in Egypt than in the other two countries, while that for the non venereal urinary and genital diseases is somewhat higher. This group of diseases together, and including diabetes, shows a mortality rate of 320 per 100,000 in Egypt as against 525 and 579 for the United States and for England and Wales, respectively, for the years indicated.

NATIONALITY AND RELIGION

The foreign born population in 1937 included 187,000 persons or 1.2 per cent of the total population, concentrated in the urban centers.

Table VII gives the distribution of deaths from cancer and from all causes by nationality for the 25 year period of 1918-1942, together with the cancer death rate per 100,000 for each nationality of foreign-born. The rates show a wide variation from 14.5 for native Egyptians to 64.8 for the Italian foreign-born. The rates for the foreign-born, with the exception of the "other foreign-born" group, are all more than twice as great as for native Egyptians.

The mortality rates for all causes, on the other hand, are high (33.8 per 1,000) for native Egyptians and low (ranging between 9.1 and 12.4 exclusive of the "other" group) for the foreign-born. The rates for the foreign-born are much as one would expect in an average European population.

The variation in cancer mortality according to nationality is in part due to differences in the age distribution. Thirty-one per cent of the foreign-born population were listed as under 20 years of age in the census of 1937, contrasted with 48 per cent for native Egyptians, whereas the percentage of the population over 50 years of age was 18 for the foreign-born as against 12 for natives. The differences in age are large, but cannot account for the higher incidence of cancer mortality among the foreign-born.

The foreign-born populations reside in the urban areas; 80.0 per cent of the total foreign population was recorded as resident in Alexandria and Cairo in 1937. A classification of the census returns by occupation shows that 39 per cent of all occupied foreign-born were employed in industry and com-

TABLE VII: CANCER MORTALITY IN THE EGYPTIAN REGISTRATION AREA, CLASSIFIED ACCORDING TO NATIONALITY; 25-YEAR PERIOD, 1918-1942

	Egyptians	Foreign-born						Total Population
		Total	British	French	Italian	Greek	Others	
Cancer deaths in 25 years	17,135	2,761	417	236	843	1,046	219	19,896
Deaths from all causes in 25 years	2,819,026	51,247	10,565	4,981	13,755	17,806	4,111	2,870,303
Cancer deaths per 1,000 total deaths	6	54	39	47	61	58	53	7
Population, Egyptian Regis. Area, in mid-period	3,336,400	225,006	34,169	24,332	52,462	76,264	38,373	3,562,000
Cancer death rate/100,000	14.5	48.8	49.7	38.8	64.8	55.1	23.4	22.3
Death rate from all causes/1,000	33.8	9.1	12.4	8.2	10.5	9.3	4.3	32.2

merce, as against 12 per cent of the occupied Egyptian population. Of the latter, 58 per cent were engaged in agriculture and fishing, as against 1.3 per cent of the foreign-born. A marked contrast thus exists between the native Egyptians and the foreign-born population; the former are rural dwellers, the latter are urban. Any comparison of mortality rates between the two groups must be made in consideration of the manner of death certification. It has been demonstrated previously that mortality reporting in Egypt is most complete in urban areas and is defective in rural ones. This fact alone is enough to account for the differences shown in Table VII.

Nationality differences in mortality in Egypt must be accepted with great caution until it is abundantly demonstrated that the system of death reporting and certification is complete.

The 19,896 deaths recorded during the period 1918-1942 are reclassified according to religion in Table VIII. Mortality rates for cancer are lowest among the Moslems, 16.3 per 100,000; next highest among the Christians, 80.5; and highest among the Jews, 187.4. In contradistinction to the rates for the foreign-born, the all-cause mortality rates vary in the same manner, though to a lesser degree.

Information is not available to give an indication of the urban-rural or the occupational distribution of the population by religion. It would appear evident, however, from a comparison of Tables VII and VIII that the Christian and Jewish populations of the latter table must include a number of areas in which mortality is exceedingly high. The foreign-born populations shown in Table VII are largely Christian or Jewish; their annual death rate is 9.1 per 1,000. Table VIII shows that the Christian and Jewish rates for all causes are 40.5 and 59.9, respectively! Further investigation must be carried out in order to clarify the results shown in Table VIII.

OCCUPATIONAL CANCER

It may be conjectured that the greater exposure of the foreign-born to the irritants and injuries of industry renders them more liable than Egyptians to attack by cancer. However, the number of foreign-born working in certain industries in which exposure to carcinogenic substances is suspected is too small to account for the results shown in Table VII. The number of such industries is quite limited in Egypt and it is only rarely, if at all, that the so-called occupational cancers are encountered in Egyptian medical practice, among either Egyptians or foreign-born.

Chimney sweep's cancer was never seen in Egypt; it has even ceased to occur in England and Wales, where it was first noticed, after the introduction of modern methods of cleaning chimneys. Cancer developing after long contact with coal and wood tars, occasionally noted in Europe and America, is unknown in Egypt. Persons trading in coal and coke are largely Egyptians and do not exceed 5,000 in number, while the number of persons preparing pitch for the maintenance of roads is also small. Malignancy developing on benign epidermal lesions of the scrotum, forearms and legs of persons engaged in paraffin works is also unknown in Egypt. Cancer in brickmakers, developing as epitheliomas in old skin lesions, is not known in Egypt, although about 7,000 persons are engaged in this occupation.

Cancer in cotton spinners, chiefly attacking workmen who run spinning machines and in the process become covered with mineral oil, is also absent in Egypt. In a cotton spinning factory in Alexandria, employing between 5,000 and 10,000 workmen under strict medical supervision, not a single case of cancer of this type was observed over a period of 12 years. The number of workmen in cotton spinning and weaving in Egypt was 28,000 in 1937; nevertheless no cases of cotton spinners' cancer have been recorded.

Miners, who do not exceed 800, petroleum refiners numbering about 1,500, and aniline dyers numbering about 5,000, also appear to be free from the cancers associated with these trades. The type of cancer found among aniline dyers is of interest to Egyptian cancerologists because of its re-

TABLE VIII: CANCER MORTALITY IN THE EGYPTIAN REGISTRATION AREA, CLASSIFIED ACCORDING TO RELIGION; 25 YEAR PERIOD, 1918-1942

	Moslems	Christians	Jews	Others	Total
Cancer deaths in 25 years	13,189	5,941	747	19	19,896
Deaths from all causes in 25 years	2,544,338	299,428	23,976	457	2,870,303
Cancer deaths/1,000 total deaths	5.2	19.8	31.2	41.6	6.9
Population, Egypt. Regis. Area in mid-period	3,244,982	295,646	16,009	5,343	3,562,000
Cancer death rate per 100,000	16.3	80.5	187.4	14.2	22.3
Death rate from all causes per 1,000	31.4	40.5	59.9	3.4	32.2

semblance to cancer of the bladder developing in connection with bilharziasis, one of the most frequently occurring types of cancer in Egypt.

X-ray cancer is also unknown in Egypt. I have not seen or heard of a single case in 20 years of practice in Egyptian hospitals, either among radiographers and physicians or among patients treated by radiotherapy, although in the provinces, x-rays are often handled by inexperienced persons.

BILHARZIASIS AND CANCER

Bilharziasis attacks from 30 to 90 per cent of the population, according to locality, and is most prevalent in rural areas, small towns and villages.

In the series of 5,924 autopsies in Kasr-el-Ainy Hospital, Cairo, 1905-1930, analyzed by Professor Sorour (16), malignant disease was found in 20.6 per cent of the cases of bilharzial bladder and in 4.4 per cent of cases of bilharzial cirrhosis of the liver. In the same series, bilharziasis and cancer were associated in 16.6 per cent of the cases of cancer of the colon. The malignant bladder cases, 85 in number, were all associated with bilharziasis.

Dr. M. Ashour (7) and Dr. Anis Onsy Bey, examining a series of pathological specimens in the Ministry of Public Health Laboratories, found in 3,180 specimens of malignant disease 117 or 3.6 per cent showing bilharzial pathological changes. The bilharzial malignancies were 8 malignant papillomas, 104 carcinomas (46 of which were vesical, or 45 per cent) and 5 sarcomas.

These and similar data have been taken by various authors as proof of a definite relationship between bilharziasis and the development of cancer in certain parts of the body, especially the bladder, prostate and colon where malignant disease is often found to develop on old-standing bilharzial lesions. Such findings are termed bilharzial cancer. The parasitic infection is not credited with provoking malignant disease, but the irritation of the parasites and their ova and embryos, with the coincident chronic sepsis is suspected of being responsible.

Dolbey and Mooro (9) studied 671 cases of cancer in various sites and found malignant disease in a large number of bilharzial bladders but none in bilharzial renal pelves, ureters and intestinal tracts. They noticed that the large intestine was often the seat of large benign growths, resulting from chronic irritation induced by the parasites, which rarely developed malignancy. They ascribed this to the acid reaction of the bowels and absence of chronic sepsis such as that found in cystitis, and suggested that the high incidence of malignancy in bilharzial bladders was due to irritation of the ova in an alkaline medium such as the stagnant urine of bilharzial cystitis. Onsy (13), Sorour (17) and Barsoum (8), however, in biopsies and autopsies found an appreciable number of cases of cancer not only in bilharzial bladders but also in bilharzial prostates, testicles, small intestine, colons, rectums, livers, uteri, ovaries, vulvas, vaginas, etc. Any explanation, therefore, of the role of bilharziasis in the development of malignant disease must not be limited to the bladder, but must include other sites as well. This fact has led to acceptance only that chronic irritative action of the bilharzial ova and embryos and of the long-

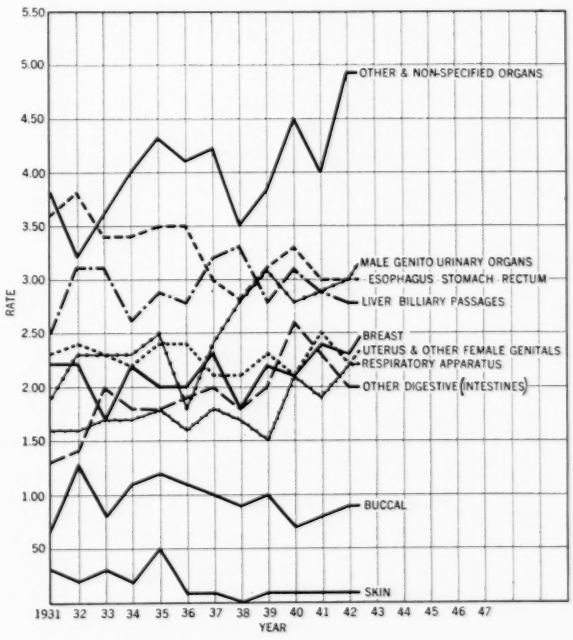


FIG. 3.—Cancer death rate per 100,000 of population in Egyptian Registration Area by organs in 12 years 1931-42.

standing inflammatory processes resulting from the parasitic infections are precursors of malignancy in bilharzial cancer.

This concept is strongly held by the majority of writers on cancer in Egypt. However, evidence of an association between bilharziasis and cancer would be difficult to avoid in a country so heavily infected with bilharziasis. Proponents of the theory of the causal relation of the disease to cancer have yet to show that the incidence of cancer is higher in a group of definite bilharzial cases than in one in which this disease is definitely absent but is similar to the first in the other pertinent factors of age, sex, race, occupation, etc. Further, unless a definite carcinogenic agent, or other predisposing factor, is proven to exist in bilharziasis, the term "bilharzial cancer" is unjustified. Investigations of this kind have not been carried out, to my knowledge, in Egypt, and the field is still quite open for research.

SUMMARY

Compared with rates for other countries, the recorded incidence of cancer mortality is low in Egypt, but death registration in the country is such that the low rate for Egypt can hardly be accepted. Before this can be done it must be abundantly demonstrated not only that all deaths within the designated registration area are reported, and the cause of death certified, but also that the certification is made as often as possible by a qualified physician with medical knowledge of the case before death. This can be accomplished in part by intensive and concerted action by the medical profession and the health authorities to secure a better registration system. Further improvement than this, however, must await a more complete development of medical care in Egypt, not only with respect to an improvement in the qualifications of the profession, but in the acceptance by the people of the medical profession as the only group qualified to diagnose and treat the ill.

It is likely that most of the differences noted between specific rates for sex, race, and areas are also due to defective reporting and certification.

Until medical practice and death registration are improved, it would appear that evidence of the actual amount of cancer in the country can best be secured by a series of well planned and conducted surveys by qualified medical investigators working in conjunction with experts in the field of statistical sampling. The promise of such surveys is great. The many contrasts of the social, occupational, racial, religious and dietary aspects of life in Egypt offer opportunity for comparisons which would yield valuable information concerning cancer.

The relation between bilharziasis and cancer is still uncertain and needs thorough and careful study, both from the point of view of the incidence of cancer in bilharzial cases and of the pathology and chemistry of the two diseases.

REFERENCES

1. Afifi, M. A. Cancer Campaign in Egypt. Proceedings of the IV International Congress of Radiology, Zurich, 1934.
2. Afifi, M. A. Study of Cancer Mortality in Alexandria in the last 15 years. La semaine Internationale pour la Lutte contre le cancer. 23-30 Nov. 1938. The Egyptian Proceedings, published by the Ministry of Public Health in 1941. pp. 235-277.
3. Annual Report of the British Empire Cancer Campaign, 1945.
4. Annual Reports on the Work of the Ministry of Public Health, 1934-1938. Egyptian Government Press.
5. Annual Reports of the Chief Medical Officer of the Ministry of Health. England.
6. Annual Returns of Births, Deaths and Infectious Diseases. 1918-1934. Statistical Department, Ministry of Finance, Egyptian Government.
7. Ashour, M. The Pathology of Cancer and Demonstration of Rare Tumor Formation Encountered in the Pathological Section, Public Health Laboratories. La Semaine de l'Egypte pour la Lutte contre le Cancer. 1941, pp. 63-71.
8. Barsoum, H. Incidence of Cancer in Egypt. La Semaine de l'Egypte pour la Lutte contre le Cancer. pp. 215-244.
9. Dolbey, R. V., and Mooro, A. W. The Incidence of Cancer in Egypt. An Analysis of 671 Cases. Lancet, 587: 1924.
10. Hoffman, F. L. Cancer in Egypt. J. Cancer Research, 14: 444-452. 1930.
11. Hoffman, F. L. The Cancer Record of 1932. Proceedings of the International Congress of Cancer Campaign. Tom. II. Madrid, 1933.
12. International Health Year Book. Vol. IV, 1930. Health Organization, League of Nations. Geneva, December, 1932.
13. Onsy, A. Rare Tumour Formations Associated with Bilharzial Infection. La Semaine de l'Egypte pour la Lutte contre le Cancer. pp. 17-47.
14. Population Census of Egypt, 1937. Census Department. Egyptian Government.
15. Roussy, G. Cancer. London: G. Harrap & Co. Ltd., 1940.
16. Sorour, M. F. An Analysis of the Results of Postmortems made in Kasr-El-Ainy Hospital, Cairo from 1905 to 1930. Personal communication.
17. Sorour, M. F. The Pathology and Morbid Histology of Bilharzial Lesions in Various Parts of the Body. Comptes Rendus de Congrès International de Med. Trop. et D'Hyg. Publie 1932. Tome IV. pp. 321-371.
18. Vital Statistics, 1935-1942. Statistical Department, Ministry of Finance, Egyptian Government.

Book Reviews

CANCER AND OCCUPATION IN DENMARK. 1935-1939. Johannes Clemmesen. Translated by Robert Fraser. Copenhagen: Nyt Nordisk Forlag—Arnold Busck. 1941. 75 pages.

In this interesting analysis of deaths from cancer in their relation to occupation occurring in Denmark during 1935 to 1939, Clemmesen points out that cancer research which was dominated during the past century by pathologists has been moved into the laboratory and has become to a large extent the domian of chemists, radiobiologists, virologists and geneticists. The important direct contact with the practicing physician has been lost thereby. Clemmesen believes this to be unfortunate since a part of the future fight against cancer will be prophylactic, and research for this reason should deal with the etiology of cancer in man. While statistical mass observations on this point are of limited value, they may provide clues to other profitable approaches to the cancer problem.

Denmark which has the highest annual mortality from cancer of all countries (14 cancer deaths per 10,000 inhabitants) offers certain advantages for statistical observations on the relation between cancer and occupation. These are, high longevity of the population permitting the unsually long latency period of cancer to take effect; a well organized and evenly distributed medical service and high medical standards; accessibility of all social classes to the same high grade specialized medical care in three modern radium centers; good communications throughout the country; homogeneity in geographic, climatic and ethnologic respects, small social and economical differences; stable and uniform occupational conditions in different parts of the country over many years; a comparatively moderate number of cases allowing a detailed analysis; death certificates filled out by physicians, based to a high proportion (about 50 per cent) on elaborate hospital and laboratory data, and representing legal documents.

Cancer mortality standardized for age groups was found to be higher in towns than in rural districts and higher in men than in women. The analysis was restricted to the male population, as great technical difficulties exist in this respect for applying such an analysis to gainfully employed women and married women. For the purpose of the analysis the cancer cases according to total number and according to organ affected were distributed among the 7 occupational groups used in the census taken in 1930 in Denmark. There was a low mortality from all forms of cancer in agriculture and a high cancer incidence in industry in persons belonging to the age group 45 to 64 years. However, the respective mortality rates from cancer in the two occupational groups ran parallel to their mortality rates from other causes. Although deaths from cancer at ages over 65 years constituted the same fraction of the total mortality in the different occupational groups, the local distribution of cancers varied in different groups. This observation suggested that in Denmark also occupation had a definite bearing on the development of cancers. While it was not possible to determine with certainty from the material available if there was a corresponding, even distribution of the cancer mortality between the different occupational groups in the age group of 45 to 64 years, it is not improbable that the latency period for the development of tumors is different in different occupations depending upon the carcinogenic potency of the injurious agent. Predisposed individuals having a tendency toward rapid cancer development were probably equally distributed among the different occupational groups. The question of the inter-relation between exogenous factors and hereditary disposition to cancer is considered of greatest importance for the possible prevention of malignant tumors.

Cancer of the stomach which accounted for 31.9 per cent of the total cancer mortality occurred with almost average frequency in the agricultural group age 45 to 64 years, while it was found at an excess of 129 per cent in the industrial group of the same age. Gastric cancer mortality was low in all age groups of the group comprising the professional and white collar class, where it stood at 57.1 per cent of the average in the age group 45 to 64. These differences were attributed to better general hygienic conditions in this class and not to the effect of some carcinogenic factors acting on the stomach of the members of the industrial group. These observations confirmed similar ones previously made in England on the uneven distribution of gastric cancer on five social groups. In England it was proposed therefore to reduce gastric cancer mortality by improving the living conditions of groups IV and V with lowest economical standards to those prevailing in groups I and II which have a low gastric cancer mortality. Unfortunately, well-to-do people were found to have a higher intestinal cancer rate than persons belonging to the socially and economically low classes. Clemmesen suggested that the differences in the local distribution of cancers of the gastrointestinal tract might be causally related to the higher amounts of foodstuffs ingested by the physically laboring classes, as there is no material differences in the quality of food consumed by all occupational groups in Denmark. Esophageal cancer incidence was high in the industrial group and low in the agricultural and white collar groups. However, by far the highest incidence of esophageal cancer was noted in persons belonging to the various alcoholic trades (waiters, restaurant and hotel owners and managers, commercial travellers, packers) confirming again in this respect previous English observations. Rectal cancers, on the other hand, were infrequent in members of the alcoholic trades, but high in warehouse men and packers. Both intestinal and pulmonary cancers were excessively frequent in the industrial group and occurred below the average figure in the agricultural group. Clemmesen stated that the tar theory of lung cancer based on the construction of tarred roads in recent years is premature, as not sufficient time

has elapsed since the introduction of such roads to have exerted any definite influence on the incidence of lung cancer.

As the discrepancy between cancer morbidity and cancer mortality rates is apt to grow with the increasing improvement of therapeutic measures, Clemmesen proposed to make cancer a notifiable disease. The information should contain exact identification of the patient and of the cancer as to site and type, histological diagnosis, occupation, and cause and place of death. Information as to the cause of death in cured cases is regarded by him as especially important. The public should be taught to avoid intimate contact not only with bacteriological dirt but also with chemical dirt, such as grease and lubricating oils. The conduct of similar surveys in other countries Clemmesen considered as valuable and indicated.

It is obvious that surveys of this nature are important and urgently needed in this country because of its leading position as an industrialized country. Information on the relation between cancer and occupation is moreover here highly defective, although the practical and scientific significance of exogenous and especially occupational agents in the genesis of cancer has become increasingly evident in recent years. There can be no doubt that a comprehensive study of the workers exposed to occupational carcinogenic agents, and affected by industrial cancers, would be of eminent value for future control not only of this type of cancer but of cancer in general.

W. C. Hueper

THE CHEMICAL KINETICS OF THE BACTERIAL CELL. C. N. Hinshelwood. Oxford: Clarendon Press. 1946, x + 284 pages. Price $6.75.

The author of this book is a recognized authority on the theoretical aspects of chemical kinetics, particularly those of gaseous reactions. In recent years he and his students have become interested in the formal analogy presented by the kinetics of bacterial growth. This volume is avowedly an attempt to ascertain whether the laws governing known chemical reactions can be used to describe quantitatively the normal growth of bacterial cells and their adaptation to various environmental influences.

From the biological viewpoint the presentation would be clearer had it begun with Chapter VII (adaptation) and continued with the remaining sections on variation, selection and cell division, ending with Chapters I to VI in which the author's views on kinetics and adaptation are developed. According to the latter, the adaptive response depends on a shift in the enzyme balance of the cell. Although mentioned in the later chapters, the alternative theories of mutation and selection receive rather cursory and inconclusive treatment. It is not our intention to enter into the merits of these rival theories here; nevertheless, it should be pointed out that important objections to the author's theory presented by recent experiments of Demerec and of Luria on penicillin and sulfonamide resistance receive no comment in spite of the fact that Demerec's paper is cited in the bibliography as a typical example of drug resistance.

The value of the book lies in its coordinated presentation of the author's own excellent quantitative data on adaptation, and the extended mathematical development of a kinetic theory derived from the concept of a shift in enzyme balance. Although in its present form the theory is not concerned with phenomena other than the growth of bacterial cells there appears to be no reason why an analogous theory could not be developed to account for certain aspects of normal and abnormal tissue cell growth. The main difficulty would appear to be in establishing whether the basic assumptions which the author postulates for his theory are really valid for any given application. Even for bacterial systems there is evidence that the mere correspondence between predicted and experimental values is not a crucial test for the validity of the theory.

Henry P. Treffers